PHOTOGRAPH THIS SHEET

AD-A184 922

DTIC ACCESSION NUMBER

LEVEL

INVENTORY 1

TESTING EXPERIMENTAL COMPOUNDS AGAINST LEISHMANIASIS IN LAB ANIMAL MODEL SYS

DOCUMENT IDENTIFICATION
SEPT 1984

This document has been approved for public release and sale; its distribution is unlimited.

DISTRIBUTION STATEMENT

ACCESSION FOR
NTIS GRA&I
DTIC TAB
UNANNOUNCED
JUSTIFICATION

BY
DISTRIBUTION /
AVAILABILITY CODES

DIST	AVAIL AND/OR SPECIAL	
A-1		

DISTRIBUTION STAMP

COPY INSPECTED

DTIC ELECTE OCT 14 1987 S D

DATE ACCESSIONED

This document has been approved for public release and sale; its distribution is unlimited.

DATE RETURNED

87 10 6 119

DATE RECEIVED IN DTIC

REGISTERED OR CERTIFIED NO.

PHOTOGRAPH THIS SHEET AND RETURN TO DTIC-FDAC

DTIC FORM 70A MAR 86

DOCUMENT PROCESSING SHEET

PREVIOUS EDITION MAY BE USED UNTIL STOCK IS EXHAUSTED.

AD _____

Testing Experimental Compounds Against Leishmaniansis in Laboratory Animal Model Systems

ANNUAL SUMMARY REPORT

Jan S. Keithly, Ph.D.

SEPTEMBER 1984

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick
Frederick, Maryland 21701-5012

Contract No. DAMD 17-83-C-3039
Cornell University Medical College
New York, New York 10021

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO. / 3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle): Testing Experimental Compounds Against Leishmaniasis in Experimental Laboratory Animal Models	5. TYPE OF REPORT & PERIOD COVERED: Annual Summary Report 12/15/83 to 11/01/84
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Jan S. Keithly, Ph.D.	8. CONTRACT OR GRANT NUMBER(s): DAMD 17-83-C-3039
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Cornell University Medical College, 1300 York Avenue, New York, New York 10021	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS: U.S. Army Medical Research and Development, Fort Detrick, Frederick, Maryland 21701	12. REPORT DATE: September 1984 13. NUMBER OF PAGES: 45
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report): Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Leishmania donovani	Amastigote	Pentostam
L. m. mexicana	Primary culture promastigote	Trypanothione
L. braziliensis guyanensis	Pentostam Dose Response	Drug delivery
BALB/c mouse	Combination Chemotherapy	
α-difluoromethylornithine (DFMO)		

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A. Pentostam Dose Response

Pentostam Dose Responses for L. donovani, L. m. mexicana, and L. b. guyanensis have been determined in BALB/c mice using amastigotes (A), primary culture promastigotes (PCP), and subcultured promastigotes (SP). In each test system, PCP are more resistant to treatment than are A or SP. This is biologically significant, since PCP are equivalent to the infective stage from sandflies. The Effective Dose (ED) for PCP in each secondary test system are:

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Leishmania subspecies	Cell Type	ED 90 PCP	ED 90 A	ED 50 PCP	ED 50 A
mexicana mexicana		1090	521	290	150
braziliensis guyanensis		90	25	21	07
donovani archibaldi		61	11	06	01

These data also show that Pentostam causes improvement and parasitological cures in WRAIR primary (hamster), secondary (BALB/c), and tertiary (owl monkey) test systems at doses and regimes equivalent to those used to treat humans. The validity of each of these models in drug screening to predict the outcome in human infections is established.

Cutaneous infections caused by L. m. mexicana are 3 to 7x more difficult to treat than are L. donovani or cutaneous L. b. guyanensis infections. This appears to be a problem in drug delivery both to tissue site and to parasite. Priority for mode of action of Pentostam and to development of novel carriers for delivery to amastigotes in cutaneous lesions is recommended.

Pentostam powder (BJ 58563) furnished by WRAIR is no longer available. A solution from the Centers for disease Control has been substituted. This presents some special problems for current data evaluation.

B. Experimental WRAIR Compounds

Seven of 10 WRAIR 1984 compounds suppress L. donovani 79 to 94% at the MTD, MTD/2, and MTD/4. It is highly probable they will be competitive with Pentostam. The remaining three compounds are variously toxic. It is too early to determine whether these three will be competitive with Pentostam.

For 6 of 7 drugs active against L. donovani, Pentostam Indices of 4.3 to 84.0 occur against L. b. guyanensis when measured as % suppression from controls. None of these drugs suppressed lesions from original size. All drugs were delivered orally as per change in protocol by 1983 COTR. We think the lack of efficacy against cutaneous lesions of 1984 drugs is a problem in drug delivery. Therefore, we are testing these 7 drugs subcutaneously as per standard protocol. One drug (BG 56256) given orally for 3 weeks in cutaneous infections is toxic, whereas the same total dose in L. donovani for one week is not. These data suggest drug accumulation and metabolism by infected tissue macrophages be explored.

C. Combination Chemotherapy of DFMO + Pentostam

Previously we had reported a synergistic effect of the polyamine inhibitor DFMO and the antineoplastic glycopeptide Bleomycin against L. donovani in mice. This was the first time in 30 years a new drug competitive with Pentostam showed efficacy against leishmaniasis. Here we report suppression of L. b. guyanensis infections in BALB/c mice by 5% DFMO alone and with 2% DFMO in combination with Pentostam after one week of treatment. The real question is whether this combination which affects essential metabolic pathways will be as synergistic as DFMO + Bleomycin, in which DNA is the ultimate target. These data suggest combination chemotherapy of DFMO with DNA intercalators and inhibitors of leishmania pathways, i.e., allopurinol, pentamidine, is a promising alternative to antimonial therapy.

D. Evaluation of L. braziliensis braziliensis

During the past two years we have shown that L. b. braziliensis M2904 and a recent human isolate have special requirements for growth and transformation in vitro. We have established experimental infections in BALB/cByJ mice in order to study properties of virulence. Because of the projected revisions in this contract for testing topical creams in hamsters against L. m. mexicana and L. b. panamensis infections, additional studies on L. b. braziliensis will

(cont.)

be carried out under separate funding. If promising compounds are identified under DAMD 17-83-C-3039, the Principal Investigator will test them against *L. b. braziliensis* at the request of COTR.

FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigators adhered to the "Guide for Care and Use of Laboratory Animals", prepared by the Committees on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

SUMMARY

A. Pentostam Dose Response

Pentostam Dose Responses for L. donovani, L. m. mexicana, and L. b. guyanensis have been determined in BALB/c mice using amastigotes (A), primary culture promastigotes (PCP), and subcultured promastigotes (SP). In each test system, PCP are more resistant to treatment than are A or SP. This is biologically significant, since PCP are equivalent to the infective stage from sandflies. The Effective Dose (ED) for PCP in each secondary test system are:

Leishmania subspecies	Cell Type	ED 90		ED 50	
		PCP	A	PCP	A
mexicana mexicana		1090	521	290	150
braziliensis guyanensis		90	25	21	07
donovani archibaldi		61	11	06	01

These data also show that Pentostam causes improvement and parasitological cures in WRAIR primary (hamster), secondary (BALB/c), and tertiary (owl monkey) test systems at doses and regimes equivalent to those used to treat humans. The validity of each of these models in drug screening to predict the outcome in human infections is established.

Cutaneous infections caused by L. m. mexicana are 3 to 7x more difficult to treat than are L. donovani or cutaneous L. b. guyanensis infections. This appears to be a problem in drug delivery both to tissue site and to parasite. Priority for mode of action of Pentostam and to development of novel carriers for delivery to amastigotes in cutaneous lesions is recommended.

Pentostam powder (BJ 58563) furnished by WRAIR is no longer available. A solution from the Centers for disease Control has been substituted. This presents some special problems for current data evaluation.

B. Experimental WRAIR Compounds

Seven of 10 WRAIR 1984 compounds suppress L. donovani 79 to 94% at the MTD, MTD/2, and MTD/4. It is highly probable they will be competitive with Pentostam. The remaining three compounds are variously toxic. It is too early to determine whether these three will be competitive with Pentostam.

For 6 of 7 drugs active against L. donovani, Pentostam Indices of 4.3 to 84.0 occur against L. b. guyanensis when measured as % suppression from controls. None of these drugs suppressed lesions from original size. All drugs were delivered orally as per change in protocol by 1983 COTR. We think the lack of efficacy against cutaneous lesions of 1984 drugs is a problem in drug delivery. Therefore, we are testing these 7 drugs subcutaneously as per standard protocol. One drug (BG 56256) given orally for 3 weeks in cutaneous infections is toxic, whereas the same total dose in L. donovani for one week is not. These data suggest drug accumulation and metabolism by infected tissue macrophages be explored.

C. Combination Chemotherapy of DFMO + Pentostam

Previously we had reported a synergistic effect of the polyamine inhibitor DFMO and the antineoplastic glycopeptide Bleomycin against L. donovani in mice. This was the first time in 30 years a new drug competitive with Pentostam showed efficacy against leishmaniasis. Here we report suppression of L. b. guyanensis infections in BALB/c mice by 5% DFMO alone and with 2% DFMO in combination with Pentostam after one week of treatment. The real question is whether this combination which affects essential metabolic pathways will be as synergistic as DFMO + Bleomycin, in which DNA is the ultimate target. These data suggest combination chemotherapy of DFMO with DNA intercalators and inhibitors of leishmania pathways, i.e. allopurinol, pentamidine, is a promising alternative to antimonial therapy.

D. Evaluation of L. braziliensis braziliensis

During the past two years we have shown that L. b. braziliensis M2904 and a recent human isolate have special requirements for growth and transformation in vitro. We have established experimental infections in BALB/cByJ mice in order to study properties of virulence. Because of the projected revisions in this contract for testing topical creams in hamsters against L. m. mexicana and L. b. panamensis infections, additional studies on L. b. braziliensis will be carried out under separate funding. If promising compounds are identified under DAMD 17-83-C-3039, the Principal Investigator will test them against L. b. braziliensis at the request of COTR.

TABLE OF CONTENTS

INDEX OF TABLES

Pages

INDEX OF FIGURES

I. Objective

To serve as a secondary screen for promising WRAIR experimental compounds against cutaneous, mucocutaneous, and visceral leishmaniasis in BALB/c mice. Specifically to test 10 compounds a year against Leishmania mexicana mexicana, L. braziliensis panamensis (or guyanensis), and L. donovani and to test 5 compounds a year against L. braziliensis braziliensis.

The original scope of this proposal was modified during its first year (1982-1983) to:

A. Determine the optimum dose and stage of parasite for screening - Parasite Dose Response
B. Determine the Pentostam Dose Response to infection
C. Screen three new WRAIR compounds
D. Optimize the Standing Operating Procedure for each model.

A summary of these results may be found in the 1983 Annual Report.

Following an evaluation of the First Annual Report 1983 in November and a Site Visit in January, 1984, the scope of this proposal was further modified chiefly due to a paucity of promising experimental drugs identified in the hamster primary test system against cutaneous and mucocutaneous leishmaniasis.

Following the Site Visit, it was recommended that:

A. Ten experimental WRAIR compounds be tested in the Leishmania donovani BALB/c model;
B. A Pentostam Dose Response (PDR) be completed for each model;
C. Combination chemotherapy using d-difluoromethylornithine (DFMO) + Pentostam against L. donovani, L. m. mexicana, and L. b. guyanensis be started as a new direction; and
D. Basic research on the L. b. braziliensis BALB/c test system continue for one year.

In addition, it was decided that new directions for the contract be identified and discussed further with the incoming COTR Major James Lovelace.

At a preliminary meeting in April, the PI presented several new directions for consideration and supporting letters of collaboration (Appendix I). These included: 1) combination of DFMO with known and promising antileishmanial drugs, 2) mode of action of Pentostam in vivo and in vitro and the use of carnitine analogs to inhibit leishmania, 3) use of iron chelators to interfer with heme-iron acquisition by leishmania, and 4) significance of iron siderophores for virulence of L. b. braziliensis subspecies. Following a meeting of WRAIR personnel in June or July, the COTR and PI will meet again to finalize the revised scope of DAMD 17-

83-C-3039 for its remaining years.

II. Background

Human leishmaniases are severely debilitating and affect about 20-100 million people (1). Their public health importance was recognized when the WHO Special Program included them among its 6 major diseases, and when the NIH-AID initiated its Collaborative Program for Training in Topical Disease. A recent report estimates 400,000 new cases yearly (2). Cutaneous disease has been a problem among U.S. Army personnel (3), Israeli troops in the Middle East (4), and recently British troops in Guyana (5). In light of events in Central and South America, and the continued interest of the United States in the security of civilians and military in this hemisphere, improving therapy for cutaneous and mucocutaneous leishmaniasis (CL and MCL, respectively) has become critical. Endemic areas of canine and human leishmaniasis probably occur within the United States (6,7).

The three drug groups for treating leishmaniasis are the pentavalent antimonials - sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime), diamidines - Pentamidine, and the macrolide antibiotics - amphotericin B. Although these are generally effective against visceral leishmaniasis, they vary in efficacy against American cutaneous and mucocutaneous leishmaniasis (8-10).

Strains of L. donovani and L. tropica resistant to pentavalent antimony and to Formycin B have recently been reported (11,12). Resistance was induced stepwise, a condition likely to occur during treatment when less than optimal dosing is used (13).

Since our last annual report, rational approaches to chemotherapy have 1) provided new lead compounds, 2) stimulated reevaluation of standard drugs, and 3) identified synergistic combinations of new and existing drugs. The incidence of American mucocutaneous leishmaniasis has also increased significantly.

A. Incidence

Since December 1983, our laboratory has been consulted in five human and two canine cases of suspected leishmaniasis. Two of the 5 human cases were isoenzyme typed as L. b. braziliensis (Peru), one as L. b. guyanensis (Ecuador), and the other two were culture negative (Ecuador, India). All but one of these patients were American. One patient negative for leishmaniasis subsequently died of fulminant histoplasmosis.

The unfortunate fact that 3/5 diagnosed cases were mucocutaneous subspecies illustrates the immediate need for improved therapy against L. braziliensis (14-17). Two patients were apparently cured using Pentostam, while one patient unsuccessfully treated with a full course of sodium stibogluconate was

subsequently cured using amphotericin B (H. Pierce-Gardner, FRS, M.D., pers. comm.). Recent cases of L. m. mexicana (14) and L. b. guyanensis (15-17) in Guyana show that American CL and MCL are no longer exotic diseases, but are health hazards for American professionals and travellers.

L. donovani was successfully cultured from one dog seen by us. Its owners were seen at the International Health Care Service, Cornell, because their 2 small children were thought at risk for L.d. infantum. The family declined to be tested or treated (H.W. Murray, M.D., pers. comm.), preferring a "wait and see" approach. Canine leishmaniasis is notoriously difficult to treat (18-19). These imported cases and those within the U.S.A. (6) are of increasing concern for the Centers for Disease Control.

B. Chemotherapy

1. Antimonials

Systemic pentavalent antimonials remain the treatment of choice for all leishmaniases. Recently, longer regimes and higher doses have improved cure and decreased relapse rates in humans (13,20). Combination of Pentostam with rifampicin or allopurinol has improved therapy of resistant cutaneous and visceral cases, respectively (21-22), and we have reported potentiation of Pentostam using BCG (Annual Report 2, DAMD 17-80-C-0016).

Previous studies using BCG against cutaneous leishmaniasis were inconclusive. Mice pretreated with BCG were better able to control L. tropica infections, as measured by reduction of lesion size and metastasis to viscera (23), but BCG was unable to alter either the course of infection or the immunological response of C3H mice to infection with L. mexicana (24).

Unlike the latter authors, we observed a marked decrease in lesion size when BCG was combined with Pentostam. Pentostam is known to accumulate both in vitro within phagolysosomes and leishmania (25), and is thought to inhibit phosphofructokinase and pyruvate kinase, two enzymes important for glycolysis (26). In kinetoplastids, these enzymes are compartmentalized into the glycosome (27). Ultrastructural evidence indicates that Pentostam treatment of L. mexicana infected hamsters causes these organelles to disappear (28). Therefore, the known specificity of Pentostam for an essential pathway localized in an organelle in leishmania, may account for its efficacy in reducing lesions when combined with BCG. Neither BCG nor levamisole alone altered L. mexicana infections (24).

The reported inability of glucantime combined with glucan to alter the course of experimental infections of L. mexicana, L. braziliensis, or L. garnamhii in C57Bl/6 mice is surprising (29). In BALB/c mice, glucantime alone suppresses lesions considerably (JSK and WH Annual Reports, 1982). Lack of potentiation may be

glucan's nonspecific, immunogenic nature (29) or to host difference, but an alternative explanation is lack of immunostimulation at the tissue site. Pretreatment or post-treatment with glucan was able to protect or cure CF1 mice infected with L. donovani (30).

Liposome encapsulation of pentavalent antimonials improves efficacy and reduces toxicity against experimental L. donovani infections (8,31), and liposomes containing lymphokine significantly suppress L.d. chagasi in C57Bl/10 mice (32). Encapsulation of amphotericin B had little or no effect upon L. tropica infections in C57Bl/6 or BALB/c mice (33). The differences in liposome treatment of visceral and cutaneous infections is probably due to difficulty in delivery to tissue sites. Therefore, improving drug delivery to tissue sites in CL and MCL deserves greater consideration

2. Heavy Metals, Cationic Drugs, and Enzyme Inhibitors

In 1984 platinum complexed to polyglutamic acid (DP-PGA) or DMSO, was reported active against bloodstream trypanosomes in vivo and in vitro (34,35), and toxicity of the heavy metal was reduced. In vivo, DP-PGA binds to the cationic surface of trypanosomes, allowing the slow release of low molecular weight platinum. The metal is then pinocytosed by the parasite (34). The use of carrier macromolecules to reduce toxicity has been employed against T. cruzi (36), and probably has implications for pentavalent antimony treatment of leishmania.

Cationic electron carriers are also active against trypanosomes and leishmania. Their mode of action appears twofold: 1) disruption of plasma membrane microtubules (37,38) and 2) inhibition of the toxic radical scavengers catalase and superoxide dismutase (39-40). The most active of these compounds are phenothiozines, a group of antineuroleptic drugs, and the dyes crystal violet and basic blue 24.

Topical or systemic administration of chlorpromazine clinically improved diffuse human L. tropica and cured experimental T. brucei infections, respectively (41,38). Although the doses of chlorpromazine used here were safe, those necessary to eradicate L. donovani in hamsters are 10x more than that tolerated by patients treated for psychiatric illness (39).

In vitro phenazine methosulfate (PMS), reversibly inhibits both SOD and catalase of L.m. amazonensis (40). Leishmania depend upon these enzymes to destroy toxic superoxide anion (O_2^-) and H_2O_2 in their cytoplasm. Crystal violet and basic blue 24 can kill leishmania, but addition of SOD or catalase does not reverse this effect (40). Therefore, these cationic carriers may bind membrane phospholipids (42) or intercalate into DNA (26). In Crithidia fasiculata, crystal violet causes genetic mutations by binding cDNA (43).

The selective action of these cationic electron carriers for kinetoplastids is remarkable. Transformation and intracellular multiplication cannot occur because pellicular microtubules are disrupted (38), and intracellular survival is impossible because toxic radicals continue to oxidize the cells (40).

Leishmania also actively synthesize enzymes which alter the environment of the phagolysosome. Amastigotes contain high cysteine proteinase activity (44). This enzyme probably raises phagolysosomal pH and prevents hydrolysis of amastigotes (44). In vitro, antipain and leupeptin inhibit the 1) growth of L. m. mexicana promastigotes, 2) transformation of amastigotes to promastigotes, and 3) intracellular replication of amastigotes in both mouse peritoneal and J774 cell line macrophages (45). In vitro, these two enzymes are competitive with pentamidine, but their in vivo activity is unknown.

3. Polyamine Inhibitors

We had mentioned previously (Final Report DAMD 17-80-C-0061, p. 15) the synergistic effect of DFMO/Bleomycin against L. donovani infections in BALB/c mice, and had recommended the testing of other known and promising antileishmanial compounds with it. During the last year, an interesting report shows that DFMO may have an additional benefit for treatment. Highly susceptible C3H mice eliminated Trypanosoma brucei rhodesiense from the blood after treatment with DFMO (46). This was concomitant with the production of antibodies which normally do not appear during infection in C3H mice. If antibodies are important in the control of MCL as recently reported (16,47), chemotherapy using DFMO may not only directly suppress infection but indirectly enhance immune function (50). Therefore, the use of polyamine inhibitors continues to be a promising avenue for chemotherapy.

4. Imidazoles and Azoles

Allopurinol, its riboside, and Formycin B have all been reported active in vitro against leishmaniasis (48). Allopurinol improves human visceral disease (21), but primates infected with MCL respond poorly to it (49). There are allopurinol-sensitive and -insensitive strains of T. cruzi (50). The basis for inhibition of leishmania purine pathways by allopurinol is well documented (48). Recently, in an elegant series of studies, allopurinol riboside monophosphate (HPPR-MP) has been shown to be 40-100x more specific for leishmania succinic-AMP-synthetase and GMP reductase than is Formycin B-MP (51). Unlike Formycin B-MP, HPPR-MP cannot bind to mammalian RNA. Therefore, HPPR has a two advantages in the treatment of leishmaniasis : 1) specificity for leishmania enzymes, and 2) inability to act as a substrate for mammalian inosine pathways (51).

The use of azoles against leishmaniasis is still controversial. Oral ketoconazole has been used at high doses once or twice daily for 3 months to treat human cutaneous and

mucocutaneous leishmaniasis (52-53). Clinical improvement was remarkable, although some patients experienced somnolence and dizziness (52). However, neither the treatment regime nor Therapeutic Index was competitive with Pentostam. Experimental infections of Trypanosoma cruzi in mice and L. donovani in hamsters have been reported to respond to high doses (200 mg/kg) of ketoconazole and related azoles (54). Using identical doses and regimes, we found no inhibition of L. donovani infections in BALB/c mice with either ketoconazole or its acid hydrolysate (Annual Report 2, DAMD 17-80-C-0016). We have also shown the inefficacy of tioconazole at its MTD against experimental infections of L. m. mexicana (Appendix II).

Although imidazoles inhibit intracellular amastigotes and promastigotes in vitro (55), recent data indicate that ergosterol biosynthesis chiefly resembles that of animals (56). This may explain why the levels of ketoconazole needed to eradicate L. tropica amastigotes from macrophages, and to suppress human experimental infections are toxic (52-53, 57). These data suggest that azoles are not a promising alternative to antimonial therapy.

III. Results

As in our revised objectives, the results will be discussed in four parts:

A. Pentostam Dose Response to L. donovani, L. m. mexicana and L. b. guyanensis.
B. Screening of 1984 WRAIR compounds against L. donovani Khartoum
C. Combination chemotherapy of DFMO and Pentostam against L. donovani, L. m. mexicana, and L. b. guyanensis
D. Evaluation of the L. braziliensis braziliensis test system

A. Pentostam Dose Response

1. Leishmania donovani Test System

The rationale for establishing Pentostam Dose Response to leishmania infections in each of the models is twofold: 1) to set the base line for establishing a Pentostam Index for experimental drugs and 2) to see whether each system is a good predictor of effective pentavalent antimony doses and regimes in humans. During the first year of this contract we showed that

a) the ED50 and ED90 for L. donovani Khartoum in BALB/cByJ mice were 1.25 and 10.1 mkd x 5, respectively;
b) single doses at 10, 20, or 80 mkd were as suppressive as multiple doses; and
c) cures occurred at 80 mkd.

These doses were considerably lower than those previously reported by us for L. donovani 1S Sudan in BALB/c mice (29 and 58 mkd, respectively), and we suggested the Khartoum and Sudan strains of L. donovani have different sensitivities to Pentostam. Both the primary hamster and secondary mouse test systems have shown single doses of Pentostam equivalent to multiple doses (Wm Hanson, pers. commun., Annual Report 1, 1983). There are still no published studies comparing single and multiple doses in non-human primates or humans.

To complete the PDR for L. donovani, we tested lower dilutions of Pentostam (10 - 0.02 mkd x 5). Here we show the ED90, 70, and 50 to be 11, 4, and 1.28 mkd, respectively, in amastigote (A) infections (Fig. 1). Effective doses to cure primary culture promastigotes (PCP) are approximately 5x higher, 61, 17, and 6.25 mkd (Table 2). Doses as low as 0.3 and 1.5 mkd suppress A and PCP infections 25% (Fig. 1). In all test systems, PCP were more resistant to Pentostam than were A (Table 2). Suppression varies from 0-23% when < one mkd is administered.

2. L. m. mexicana Test System

Previously in L. m. mexicana infections we had shown that:

a) 400 mkd Pentostam suppressed lesions 85%, and apparently cured these mice.
b) percent suppression was directly related to treatment regime - a full course of 15 days was necessary,
c) male mice were more resistant to Pentostam treatment than were females.
d) at 400 mkd liver and spleen weights of treated mice were significantly larger than untreated controls,

In a series of three experiments to determine the PDR for A, PCP, and subcultured promastigote (SP) L. m. mexicana infections in our secondary test system (Fig. 2), we show that:

a) cures were achieved only at 660 mkd x 15, the LD50 for Pentostam (Table 1A);
b) amastigotes survive at the maximum tolerated dose (MTD = 330 mkd x 15) to an absolute number of 12 $\pm$ 29 cells/mg tissue as measured by serial µL dilutions (Table 1B, last column);
c) the ED90 and 50 of Pentostam for L. mexicana infections varies with cell type (Table 2). Primary culture promastigotes (PCP) are 2-3x more resistant to treatment than A or SP (Fig. 2);
d) Pentostam has no significant effect upon liver, spleen, or body weights of mice at any doses tested (data not shown);
e) lesions ulcerated in 50-100% of untreated and low dose treated mice (<41 mkd);
f) metastasis to viscera never occurred in Pentostam-treated mice; whereas it usually did in untreated

controls.

These data show that although L. mexicana infections may be suppressed 25 - 80% by Pentostam, parasitological cures are rarely achieved, and then only at toxic levels (Tables 1A and 1B).

3. L. b. guyanensis Test System

Previously we had shown that amastigotes of L.b. panamensis or L. b. guyanensis were suppressed 77 or 91%, respectively, when Pentostam was administered for three consecutive weeks SC at 416 Sb^v mkd (Annual Report, 1982). Neither of these doses was curative, and eight weeks post-infection (2 weeks after last treatment) all lesions were culture positive. There was no apparent difference in suppression, healing, or cure when ulcerated versus closed lesions were evaluated.

In a series of three experiments to determine the PDR for A, PCP, and SP of L. b. guyanensis (Fig. 3), we show that:

a) cures were achieved at >250 mkd x 15 as measured by culturing (Table 3). This is 3x less than that required to cure L. m. mexicana infections.
b) the ED90 and 50 of Pentostam for L. b. guyanensis infections also varied with cell type (Table 2). PCP were 3-4x more resistant to therapy than were A or SP (Fig. 3).
c) the ED90 for L. b. guyanensis was one to 2 logs lower than that for L. m. mexicana (Table 2),
d) as in L. m. mexicana infections, Pentostam had no significant effect upon liver, spleen, or body weight dose tested (data not shown),
e) metastasis to viscera did not occur.

These results suggest that Pentostam cures cutaneous infections caused by a mucocutaneous species, and at doses 3x lower than those suppressing CL. The significance of this finding for patients with MCL is unknown, but suggests that early Pentostam therapy of skin test- or seropositive patients might help prevent disfiguring MCL.

B. Experimental WRAIR Compounds

1. L. donovani Test System

Previously, none of the 15 experimental WRAIR compounds tested by us were competitive with Pentostam (Annual Report 1, 1983; Final Report, 1982).

Five 8-aminoquinolines, two imidazoles, and the antimalarial primaquine phosphate were tested for efficacy as antileishmanial agents. All had low Therapeutic and Pentostam Indices, and three of the aminoquinolines caused mild to moderately toxic symptoms

including i)bleeding at injection site, (ii tachynea, iii) internal hemolysis, and iv) hyperactivity. These are known side effects of aminoquinolines (58).

The rational for testing aminoquinolines was that those groups with substitutions at the 3 and 5 methyl groups (lepidines) are especially active against leishmania in vivo (59) and in vitro (60). Although their mode of action is not yet understood, it is suggested that they interfer with mitochondrial respiratory ubiquinones (26). The 8-aminoquinoline moxipraquine (61) was suppressive against T. cruzi, L. mexicana and L. braziliensis as long as treatment was applied, but cures were never achieved. This compound reached clinical trials before it was discovered to be teratogenic in rats and rabbits. At that time its development as an alternative to antimonials was discontinued. To date then, none of the aminoquinolines tested have had Therapeutic Indices competitive with pentavalent antimonials.

This year, 7 of 10 WRAIR-84 experimental compounds have been tested against L. donovani (Table 4). All are well-tolerated at levels active against Plasmodium spp. (Table 5), and all are 74-94% suppressive against L. donovani in our secondary test system (Table 4). Since these drugs were tested at the Maximum Tolerated Dose (MTD) and two-fold dilutions lower (MTD/2 and /4), it is extremely likely the ED 90 and 50 of these drugs will be competitive with Pentostam. Currently, we are retesting these seven drugs at lower doses. Of the remaining three compounds, BK 63407 is extremely toxic at very low doses (Tables 4 and 5).

2. L. m. mexicana Test System

Based upon the suppression observed in our L. donovani model, we are currently testing the seven experimental WRAIR drugs against cutaneous infections as per our standard protocol.

The first series of tests suggest that none of the 84 WRAIR compounds is effective against L. m. mexicana infections (Table 6), at doses which suppress L. donovani >90% (Table 4). For 6 of 7 drugs, Pentostam Indices (PI) are excellent when measured as % suppression from infected controls (4.3 - 84 PI, Table 6 last column). However, there is no suppression of lesions from original size (Table 6, Column 3). Drug G (BG56256) is also toxic for L. m. mexicana infected mice at doses safely used to treat L. donovani infections (Table 6). We had previously observed this phenomenon in L. mexicana infections (Annual Report 1, DAMD 17-83-C-3039), and still have no explanation for it. Perhaps this drug is photosensitive, or pentostam accumulation and metabolism within infected tissue macrophages yields toxic metabolites and/or an exaggerated host response. This altered toxicity of some drugs in cutaneous infections should be explored.

3. L. b. guyanensis Test System

As per our revised protocol, only 1984 experimental drugs showing promise against L. m. mexicana will be further tested against L. b. guyanensis. So far, none of this year's compounds appear effective against CL (Table 6), and have not then been tested against L. b. guyanensis.

C. Combination Chemotherapy of DFMO +Pentostam

Previously we had reported a synergistic effect of the polyamine inhibitor DFMO and the antineoplastic glycopeptide Bleomycin against L. donovani infections in mice (Annual Report 2, 1982, DAMD 17-80-C-0061). This was the first time in 30 years a new drug competitive with Pentostam showed efficacy against leishmaniasis. Because DFMO is parasite specific, non-toxic, and easy to deliver, we are currently testing it in combination with Pentostam solution (Burroughs-Wellcome, Beckenham, Kent, England, 100mg/ml) against L. donovani and L. m. mexicana and L. b. guyanensis infections.

1. L. donovani Test System

Three days prior to infection mice are dosed with 0, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0% DFMO in their drinking water (total = 10 days). On day 3, Pentostam is given subcutaneously at its ED 25, 50, or 90. One percent DFMO + 3 mkd Bleomycin or Pentostam alone at 80 mkd (curative dose) serve as positive controls. Mice sham-treated with saline serve as negative controls. As a further test, mice will be identically treated one week after infection is initiated.

2. L. m. mexicana Test System

Mice have been infected with and will be treated with DFMO + Pentostam as per our standard protocol (Annual Report 1, 1983). DFMO concentrations are identical to those in the visceral test system, but Pentostam doses are 160, 80, and 40 mkd x 15, the ED50, 25, and 11 for SP L. m. mexicana infections, respectively.

In a pilot experiment, suppression by Pentostam of lesions was not enhanced when combined with 0.5 - 3% DFMO in the drinking water (Table 7). Based upon results with L. b. guyanensis (C.3.), however, this experiment should be repeated with higher doses of DFMO.

3. L. b. guyanensis Test System

Using our standard protocol (Annual Report 1, 1983), we are currently treating mice with 0 - 5% DFMO and Pentostam at 6.25, 12.5, 25, and 50 mkd x 15, the ED 50, 75, 90 and 100, respectively. One and 3 percent DFMO + 3 mkd Bleomycin, or Pentostam alone at 50 mkd serve as positive controls. Mice sham-treated with water serve as negative controls. After only one week, the lesions of mice infected with L. b. guyanensis which had been treated with 5% DFMO alone were suppressed 46% from control and

40% from original lesion sizes, respectively (Table 8). However, Pentostam alone (positive control) did not suppress lesion sizes at any dose tested (Table 8, Column 1). This drug was furnished by the CDC. Using Pentostam powder provided by WRAIR, these doses should have been approximately 25% suppressive after one week (Annual Report 2, DAMD 17-80 C-0061, 1982). This suggests that a PDR for this commercial preparation in each of the test systems is necessary if WRAIR can no longer provide the powder (J. Berman, pers. comm.). It may also account for the poor results observed in our L. m. mexicana DFMO + Pentostam experiment (Table 7). Bleomycin alone suppressed L. b. guyanensis infections 25%. Its activity is not enhanced when combined either with 1 or 3% DFMO. Lesions of all mice treated with >2% DFMO and/or 12.5 mkd Pentostam improved as measured by scabbing. Two of 15 mice receiving 5% DFMO died, and all showed weight loss of 1-3 g during the first week. Therefore, at higher doses of DFMO, some toxicity is noted. Most mice stabilized after the first week of treatment.

D. Evaluation of L. braziliensis braziliensis Test System

Previously we had suggested that lack of lesion development upon initial infection with L. b. braziliensis was due in part to sex of host and conditions of medium. Subsequently (Annual Report 1983), we showed that

1. blood agar overlaid with CSM or HBSS allowed transformation of amastigotes;
2. growth was equivalent in each;
3. 7-9 week old male mice infected with $>10^7$ promastigotes were 100% infected;
4. amastigotes from mouse lesions were more infective for mice than those from hamsters;
5. tail base was superior to footpad infections.

During the second year of this contract we have shown that:

1. mouse lesions are a better source of amastigotes for drug screening as measured both by transformation and growth in culture (Table 9), and by lesion size and % infection in mice (Tables 10);
2. a solid substrate (plain agar) is necessary for amastigote to promastigote transformation (Table 9);
3. serum [Schneider's drosophila medium + 15% v/v heat inactivated fetal bovine serum (SDM+) v/s Hanks' balanced salt solution (HBSS) or SDM] is essential for promastigote growth (Table 9);
4. heme is necessary for infectivity (BA v/s SDM+, Table 10);

We have recent evidence that hamsters are better hosts for initial recovery of recent human isolates than are BALB/cByJ mice. In February of this year, we cultured L. b. braziliensis MHOM/PR/84/CUMC2 from a patient. Primary and subcultured

promastigotes from the initial isolate were cultivated either in BA/SDM+ or SDM+, and were inoculated intradermally at the optimum dose into mice or hamsters. Only the hamsters developed lesions. Several of these have typical nasal MCL, one is completely depilated and one died recently of metastasis to viscera. A recent isolate may be essential for developing reliable lesions of L. b. braziliensis for screening. We now have that isolate. Recently, we have shown that cloning can yield highly virulent L. b. guyanensis which are genetically stable. Cloning this recent isolate of L. b. braziliensis would offer another alternative for developing a rapid, reliable infections for drug screening in mice or hamsters.

IV. Discussion and Conclusions

A. Pentostam Dose Response

As mentioned previously, the rationale for establishing Pentostam Dose Response to leishmania infections in each of the experimental animal test systems is twofold: 1) to set the base line for establishing a Pentostam Index for experimental drugs and 2) to see whether each system is a good predictor of effective pentavalent antimony doses and regimes in humans.

1. Visceral Test System

To date, meglumine antimoniate (26.3% Sb^V) in both the hamster and owl monkey primary and tertiary test systems suppress and/or parasitologically cure L. donovani Khartoum infections in an 8 to 10 day screen at 90 - 104 mkd (2,34). Using an 8 day screen in our secondary BALB/c test system, we obtain identical results using 80 mkd. These data agree remarkably with those of Stauber (65) who first established the 8-day screen in hamsters (ED 90 = 94 mkd). They also show that whether BALB/c mice, outbred hamsters, or owl monkeys are used, Pentostam suppresses L. donovani at identical doses. Therefore, these data indicate that each animal can serve as a valid model in which to screen experimental drugs.

How well do these models predict the human response to Pentostam treatment? Two recent human studies using Pentostam against Kenyan and Indian kala-azar show that these test systems are good predictors of curative doses and regimes used for human infections (13,20). As in animal models, improvement was noted in 80-95% of the patients by the end of the first week (20). Parasitological cures were achieved in 98% of Indian patients and 88% of Kenyan patients treated more than 20 and 30 days, respectively (13,20). Similar cure rates were noted 15 days after treatment in Chinese patients (67). The total doses achieving cures in both humans and BALB/c mice, hamsters, or owl monkeys is 400 to 660 mkd. Therefore, in all animal test systems Pentostam produces parasitological cures at levels and regimes which cure patients.

In each of the human studies a few patients did not respond to pentavalent antimonials (13,20,67). In Kenya, these patients were eventually cured by combining Pentostam with allopurinol (20), and in India or China by combining the drug with Pentamidine (13,67). These data indicate that combination chemotherapy inhumans is a valid alternative to antimony therapy alone.

Differences in human sensitivity to antimony therapy have been chiefly attributed to the parasite (11). A Kenyan strain of L. donovani became senstive to antimony after passage in hamsters. The authors postulate this may be due either to a basic change in the parasite or to selection of a subpopulation sensitive to antimony (11). There is an alternate plausible explanation. The metabolism of Pentostam in hamsters may produce different kinds and amounts of metabolites than do humans. This phenomenon accounts for the difference in activity of allopurinol and its riboside against L. donovani in hamsters and humans (52).

Unlike humans who required sustained treatment with pentavalent antimonials (13,20a), PDRs in both hamsters and mice have shown they can be cured with a single dose (68, Annual Report 1, 1983).

Although absolute amounts of antimony in animal tissues have been measured (69), nothing is known about its active metabolites. We do know that antimony preferenti lly accumulates within the liver and spleen (69), and that ^{125}Sb sodium stibogluconate accumulates within amastigotes in vitro (27). Direct inhibition of the glycolytic enzymes of leishmania may occur, as these enzymes are compartmentalized into a special organelle, the glycosome (29). Some evidence suggests that Pentostam localizes in this organelle (31). The active metabolites of Pentostam are completely unknown, but are assumed to be trivalent compounds, which interfer with phosphofructokinase (PFK) and pyruvate kinase (28). In leishmania, however, PFK is not under strict regulation (52). This may mean that Pentostam has more than one mode of action in order to be so effective against these parasites.

Rational approaches to chemotherapy of leishmaniasis in the last few years and months have identified several enzyme systems specific for leishmania which could be additional, potential targets for Pentostam:

(1. Purine salvage - HPPRase (28)
(2. Toxic radical scavengers - FeSOD (66)
(3. Polyamine biosynthesis - ODC (67)
(4. Glutathione reductase (68,69)

Of these four, the latter two offer the best potential target for Pentostam. Glutathione (GSH) and glutathione reductase (GSSH) contain -SH groups, the proposed site of inhibition by heavy metals (28). Specific inhibition of GSH synthesis by D,L-Buthionine-SR-Sulfoxamine (BSO) has already been used to cure trypanosomiasis in mice (68). Recently, a unique cofactor

essential for GSH-GSSH conversion, "trypanothione", has been isolated and purified from leishmania and trypanosomes (69). This cofactor is unique because it consists of GSH + glycine, cysteine, and spermidine. Arsenicals and pentostam may exert activity against the -SH groups of this essential cofactor (69). Structurally, Pentamidine resembles spermidine. Therefore, it may specifically prevent trypanothione biosynthesis, by substituting for spermidine.

With this new information, one could predict why combination therapy of DFMO + Pentostam should be synergistic:

(a. DFMO acts at the rate limiting step of polyamine biosynthesis. Its overall effect is to decrease putresine, spermidine, and spermine (67).

(b. Spermidine is necessary for the biosynthesis of trypanothione an essential cofactor for GSH reduction.

(c. Pentostam binds -SH groups

(d. By depleting polyamines essential for cell division, protein synthesis, and cofactor biosynthesis using DFMO, and by binding -SH groups of GSSH necessary to scavenge radicals using Pentostam, parasites should be killed more rapidly.

Antimonials are not only our best drugs, they appear to be highly selective and specific for leishmania. For this reason, we recommend the mode of action of Pentostam be given high priority by WRAIR.

We think the difference in efficacy of Pentostam against visceral, cutaneous, and mucocutaneous infections caused by PCP is biologically significant. These cells are equivalent to those transmitted by flies to humans in natural infections. There are two points we wish to make. The higher doses of Pentostam and longer continuous regimes recently proposed by the WHO and supported by researchers in India are firmly supported by our experimental data. Secondly, the physiological and biochemical bases for differences between PCP and A or SP should be given utmost priority and thoroughly explored. This for the long range goals of both improved chemotherapy by mode of action studies, and vaccine production by identifying the virulence factor(s) in PCP.

2. Cutaneous and Mucocutaneous Test Systems

Unlike L. donovani, there are no carefully controlled human studies with which to compare our PRDs in hamsters and mice against L. m. mexicana or L. b. guyanensis infections. However, the immune response of BALB/cByJ to L. b. guyanensis infections

(16) is very much like that reported for human MCL infections (47). Antibody plays a more important role, than does cell-mediated immunity, and immunosuppression of this response is correlated with metastasis to far sites leading to mucocutaneous disease (16, 70). Therefore, these data suggest that the BALB/cByJ mouse is a good predictor for behavior of MCL in humans, and that the effect of Pentostam and experimental drugs against MCL in this model is valid. It also suggests that conclusions about model validity based upon host response to other species, i.e. L. tropica, is fallacious and counterproductive. There are no data on immune resonse of inbred hamsters to any of these infections.

We recommend that WRAIR keep each of its test systems for visceral, cutaneous, and mucocutaneous leishmaniasis in BALB/c mice.

B. Experimental WRAIR Compounds

The rationale for testing primaquine phosphate, 8-aminoquinolines, and ketoconazole against experimental infections of leishmania arose from a real need to identify drugs effective against cutaneous and mucutaneous leishmaniasis in the Panama Canal Zone. Known and experimental drugs are equally effective against human and experimental visceral leishmaniasis. To date, however, there are no drugs, including antimonials, which unequivocally cure L. mexicana or L. braziliensis infections.

Unlike the 83-WRAIR drugs previously tested, 7 of ten received this year are active against L. donovani at doses competitive with Pentostam (Tables 4,5). Efficacy of 6 of these 7 against L. m. mexicana infections is remarkable when measured as % suppression from control, generating Pentostam Inceces of 4.3 - 84 (Table 6, last column). However, when % of original lesion size is measured, none of these compounds is suppressive (Table 6, column 3). Since we are using the MTD and 2x serial dilutions, it is doubtful that new lead compounds will be identified from this 84-series against Cl or MCL. However, the potent efficacy of these same drugs against visceral infections, suggests that drug delivery may be the major reason for poor efficacy against cutaneous and mucocutaneous infections.

Previously, we showed reduction of Sbv to its active metabolites occurred systemically. When Pentostam is delivered SC, it cures L. donovani and suppresses L. mexicana and L. braziliensis infections >85% (Annual Report 2, DAMD 17-80-C-0061). In this series of experiments, drugs were delivered orally, as per a change in protocol by COTR in 1983. If these new drugs require slow delivery from a cutaneous depot, as Pentostam does against L. m. mexicana and L. b. guyanensis, then we may be missing their activity. For this reason, we plan to retest the three most active 84 WRAIR drugs using SC injections as per our standard protocol.

We do not yet know what the classes of compounds are represented in the 84 WRAIR series. Their potency suggests DNA or specific enzyme inhibitors. These compounds were chosen because of their effectiveness in the primary hamster screen by 1984 COTR Lovelace. Since some of the most promising compounds were unavailable, substitutions were made in the last several sent. This may account for their greater toxicity.

The toxicity of BG 56256 in L. m. mexicana infections at doses safely used against L. donovani is intriguing. We have noticed this phenomenon before, and recommend it be explored. Is the immune response to tissue infections somehow potentiating drug toxicity? Is the drug photosensitive? If information on the drug were available, perhaps some of these questions could be answered.

We are currently evaluating lower doses of the 7 active WRAIR compounds in our L. donovani test system.

C. Combination Chemotherapy of DFMO + Pentostam

The rationale for testing DFMO with Pentostam is based upon the specific action of DFMO upon the rate limiting step in polyamine biosynthesis of kinetoplastids, its low toxicity, and its ease of delivery in drinking water. Although the mode of action of Pentostam is incompletely known, evidence suggests several specific essential enzyme targets in kinetoplastid metabolism (Section IV. A.). Therefore, if these two drugs could act synergistically to specifically interfer with the essential pathways of leishmania, complete cures of CL and MCL at lower doses and more reasonable regimes might be realized. Based upon preliminary results for DFMO + Bleomycin in the L. donovani model (Annual Report 1, 1983), and the newly proposed sites of Pentostam activity, we would expect the combination of DFMO and Pentostam to be effective.

The real question is whether the combination of two drugs affecting essential metabolic pathways in leishmania (DFMO + Pentostam) is likely to be as synergistic as DFMO + Bleomycin in which DNA is the ultimate target. If the essential cofactor trypanothione depends solely upon polyamine biosynthesis for its spermidine moiety, and the reduction of GSH to GSSH upon trypanothione, then specific, enchanced killing of leishmania may occur at levels equivalent to DFMO + Bleomycin. However, alternate means of obtaining polyamines or of reducing GSH, albeit at less efficient rates, may exist in leishmania. If this is true, then less dramatic results may be expected by combining DFMO with Pentostam.

Our data for L. b. guyanensis suggest that high concentrations (5%) of DFMO alone may suppress cutaneous lesions. It also indicates that delivery of DFMO to intracellular amastigotes at a tissue site readily occurs. The lack of effect against L. m. mexicana infections in tissue sites may be due to differences in:

(a. uptake of DFMO by these two species,
(b. delivery within the phagolysosome, or
(c. distribution to macrophages within the lesion.

The first of these is easily tested. In collaboration with Dr. C.J. Bacchi, we are currently testing the in vitro uptake of labelled DFMO by L. m. mexicana, L. b. guyanensis, and L. donovani. Phagolysosomes containing amastigotes of L. m. mexicana are much larger than those containing L. b. guyanensis (JSK and MR, unpublished observations). In collaboration with Dr. M. Rabinovitch and Dr. Owen Griffith, we are currently testing the uptake of labelled Pentostam and DFMO into these organelles. The third possibility is more difficult to test. In BALB/cByJ mice, lesions of L. mexicana resemble large histiocytomas whereas those of L. b. guyanensis are spreading ulcerative lesions with raised borders. The number of amastigotes per mg tissue is 2 -3 logs less in the latter, as measured by ul dilutions. Delivery to infected macrophages within the histiocytoma will probably require a novel carrier system. As previously mentioned, liposomes do not appear to enhance drug delivery in either L. mexicana or L. tropica infections (29,33). The enhanced activity of platinum bound to PGA or DMSO, and daunorubicin against T. brucei and T. cruzi infections, respectively (34-36), suggests these carriers be tested against CL and MCL. Or, combination of antipain and leupeptin with one of the phenothiozines or dye electron carriers might be useful. For CL, any of the new leads mentioned in this report using various routes of delivery could be explored.

Problems

As mentioned previously (Section III, C. 3), in all DFMO + Pentostam experiments, Pentostam (100 mg/ml) furnished by the CDC was substituted for BJ 58563 when this compound became unavailable from WRAIR. All PDRs against L. donovani, L. b. guyanensis, and L. m. mexicana in our secondary test system were determined using this powder. We had previously shown (Annual Report 1, DAMD 17-80-C-0061) that the solution provided by the CDC was less active than the provided by WRAIR. Three alternatives have been suggested for solving this problem:

(1. obtain more BJ 58563,
(2. repeat all PDRs using CDC Pentostam or
(3. switch all testing to glucantime as standard.

Since the proposed revision of this contract for the third year includes testing of topical creams in a hamster model, while completing DFMO + Pentostam in BALB/c mice, we recommend enough BJ 58563 be provided to complete the latter and that glucantime be used in the hamster experiments. This would be time and cost efficient.

D. L. b. braziliensis Test System

During the past two years under DAMD 17083-C-3039, we have shown that L. b. braziliensis M2904 and a recent human isolate have special requirements for growth and transofrmation in vitro. We have established experimental infections in BALB/cByJ mice in order to study properties of virulence. Because of the projected revisions in this contract for testing topical creams in hamsters against L. m. mexicana and L. b. panamensis infections, additional studies on L. b. braziliensis will be carried out under separate funding being requested by the Principal Investigator (PI) to the National Institutes of Health and to the National Science Foundation. If promising compounds are identified under the current contract, the PI will test them against L. b. braziliensis in BALB/cByJ mice at the request of COTR Lovelace.

V. Literature Cited

1. Mahmoud, A.A.F. and K.S. Warren. 1977. Algorithms in the diagnosis and management of exotic diseases. 24. Leishmaniases. J. infect. Dis. 136: 160-163.

2. Scientific Working Groups of the UNDP/World Bank/WHO, Third Annual Report. 1980.

3. Takafuji, E.T., L.D. Kendricks, J.L. Daubek, K.M. McNeil, H.M. Scagliola, and C.L. Diggs. 1980. Cutaneous leishmaniasis associated with jungle training. Amer. J. trop. Med. Hyg. 29: 516-520.

4. Naggan, L., A.E. Gunders, R. Dizian, et al.. 1970. Ecology and attempted control of cutaneous leishmaniasis around Jericho, in the Jordan Valley. J. infect. Dis. 212: 427-432.

5. Low-A-Chee, R.M., P. Rose, and D.S. Ridley. 1983. An outbreak of cutaneous leishmaniasis in Guyana: epidemiology, clinical and laboratory aspects. Ann. trop. Med. Parasitol. 77: 255-260.

6. Andersen, D.C. et al.. 1980. Endemic canine leishmaniasis. Vet. Pathol. 17: 94-96.

7. Shaw, P.D., L.T.Quigg, D.S. Allain, D.D. Jouranck, and G.R. Healy. 1976. Autochthonous cutaneous leishmaniasis in Texas. Amer. J. trop. Med. Hyg. 25: 788-796.

8. Walton, B.C. et al.. 1968. Treatment of American cutaneous leishmaniasis with cycloguanil pamoate. Amer. J. trop. Med. Hyg. 17: 814-818.

9. Belfast, E. and R. Medina. 1971. Treatment of the mucosal form of American leishmaniasis. Derm. Venez. 10: 1121-

1147.

10. Rocha, R.A. et al.. 1980. Apparent Glucantime failure in five patients with mucocutaneous leishmaniasis. J. trop. Med. Hyg. 83: 131-139.

11. Hanson, W.L., L.D. Hendricks, D.E. Davidson, Jr., W.T. Hockmeyer, and W.L. Chapman, Jr. 1982. Relative insensitivity of a Kenyan strain of Leishmania donovani to pentavalent antimony therapy in hamsters. J. Parasitol.

12. Rainey, P. and D.V. Santi. 1983. Formycin B resistance in Leishmania. Biochem. Pharmacol. 33: 1374-1377.

13. Thakur, C.P., M,Kuman, S.K. Singh, D. Sharma, U.S. Prasad, R.S.P. Singh, P.S. Dharwan, and V. Achari. 1984. Comparisons of regimes of treatment with sodium stibogluconate in kala-azar. Brit. Med. J. 288: 8895-897.

14. Murray, H.W., J.S. Keithly, and A. Acosta. 1983. Reversible defects in antigen specific lymphocyte functions in human cutaneous leishmaniasis. Clin. Res. 31: 37a (Abstr).

15. Evans, D.A., S.M. Lankam, C.I. Baldwin, and W. Peters. 1984. The isolation and isoenzyme characterization of Leishmania braziliensis subsp. from patients with cutaneous leishmaniasis acquired in Belize. Trans. R. Soc. trop. Med. Hyg. 78: 35-42.

16. Galvao-Quintao, L., V. Colomer-Gould, N. Nogueira, and J.S. Keithly. 1985. Characterization of virulent and avirulent clones of Leishmania braziliensis guyanensis. (In preparation).

17. Colomer-Gould, V., L. Galvao-Quintao, J.S. Keithly, and N. Nogueira. 1985. Amastigote and promastigote surface antigens of Leishmania donovani, L. m. mexicana, L. m. amazonensis, L. tropica, and L. braziliensis guyanensis. J. Immunol. (In preparation).

18. Chapman, W.L., Jr., W.L. Hanson, C.R. Alving, and L.D. Hendricks. 1984. Antileishmanial activity of liposome-encapsulated meglumine antimoniate in the dog. Amer. J. Vet. Res. 45: 1028-1030.

19. Keenan, C.M., L.D. Hendricks, L. Lightner, H.K. Webster, and A.J. Johnson. 1984. Visceral leishmaniasis in the German Sherpherd dog. I. Infection, clinical disease, and clinical pathology. Vet. Pathol. 21: 74-79.

20. Rees, P.H., P.A. Kager, B.T. Wellde, and W.T. Hockmeyer. 1984. The response of Kenyan kala-azar to treatment with sodium stibogluconate. Amer. J. trop. Med. Hyg. 33: 357-361.

21. Tha, T.K. 1983. Evaluation of allopurinol in the treatment of Kala-azar occurring in North Bihar, India. Trans. R. Soc. trop. Med. Hyg. 77: 204-207.

22. Pareek, S.S. 1984. Combination therapy of sodium stibogluconate and rifampicin in cutaneous leishmaniasis. Pharmacol. Ther. 70-71.

23. Weintraub, J. and F.I. Weinbaum. 1977. The effect of BCG on experimental cutaneous leishmaniasis in mice. J. Immunol. 118: 2288-2290.

24. Grimaldi, G.F., P. Moriearty, and R. Hoff. 1980. Leishmania mexicana in C3H mice: BCG and levamisole treatment in established infections. Clin. exp. Immunol. 41: 237-242.

25. Croft, S.L., K.D. Neame, and C.A. Homewood. 1981. Accumulation of 125Sbv sodium stibogluconate by Leishmania mexicana amazonensis and L. donovani in vitro. Comp. Biochem. Physiol. 68c: 95-98.

26. Gutteridge, W.E. and G.H. Coombs. 1977. Biochemical mechanisms of drug action. University Park Press, Baltimore, MD, chapter 9, pp. 121-126.

27. Opperdoes, F.R. and P. Borst. 1977. Localization of nine glycolytic enzymes in a microbody-like organelle in Trypanosoma brucei: the glycosome. FEBS Lett. 80: 360-364.

28. Keithly, J.S. and S.G. Langreth. 1983. Inefficacy of Metronidazole against Leishmania donovani, L. mexicana, and Trypanosoma brucei brucei. Amer. J. trop. Med. Hyg. 32: 485-496.

29. Avila, J.L, F. Biondo, H. Manzon, and J. Convit. 1982. Cutaneous leishmaniasis in mice: resistance to glucan immunotherapy either alone or combined with chemotherapy. Amer. J. trop. Med. Hyg. 34:53-59.

30. Cook, J.A., T.W. Holbrook, and B.W. Parker. 1980. Visceral leishmaniasis in mice: Protective effect of glucan. J. Reticuloendothel. Soc. 27: 567-573.

31. Chapman, W.L., Jr., W.L. Hanson, and L.D. Hendricks. 1983. Toxicity and efficacy of meglumine antimonate in the owl monkey (Aotus trivirgatus). J. Parasitol. 69: 1176-1177.

32. Reed, S.G., M. Barral-Netto, and J. Inverso. 1984. Treatment of experimental visceral leishmaniasis with lymphokine encapsulated liposomes. J. Immunol. 132: 3116-3119.

33. Panosian, C.B., M. Barza, F. Szoka, and D.J. Wyler. 1984. Treatment of experimental leishmaniasis with liposome intercalated amphotericin B. Antimicrob. Agents Chemother. 25:

655-656.

34. Meshnick, S.R., D. Brown, and G. Smith. 1984. Capacity of a cis-diamiminoplatinum [II]-polyglutamic acid complex to cure Trypanosoma congolense infections in mice. Antimicrob. Agents Chemother. 25: 286-288.

35. Farrell, N.P., J. Willamson, and D.J. M. McLaren. 1984. Trypanocidal and antitumor activity of platinum-metal and platinum-metal drug dual function complexes. Biochem. Pharmacol. 33: 961-971.

36. Williamson, J. and T.J. Scott-Finnigan. 1981. Trypanocidal activity of daunorubicin and related compounds. Nature (London) 292: 466-469.

40. Hammond, D.J., B. Cover, and W.E. Gutteridge. 1984. A novel series of chemical structures active in vitro against the trypomastigote form of Trypanosoma cruzi. Trans. R. Soc. trop. Med. Hyg. 78: 91-95.

38. Seebeck, T. and P. Gehr. 1983. Trypanocidal action of neuroleptic phenothiozines in Trypanosoma brucei. Mol. Biochem. Parasitol. 9: 197-208.

39. Pearson, R.D., A.A. Manian, D. Hall, J.L. Harcus, and E.L. Hewlett. 1984 Antileishmanial activity of chlorpromazine. Antimicrob. Agents Chemotherap. 25: 571-574.

40. Nabi, Z.F. and M. Rabinovitch. 1984. Inhibition by superoxide dismutase and catalase of the damage of isolated Leishmania mexicana amazonensis by phenazine methosulfate. Mol. Biochem. Parasitol. 10: 297-303.

41. Henriksen, T.H. and S. Lende. 1983. Treatment of diffuse cutaneous leishmaiasis with chloropromazine ointment. The Lancet i. 126.

42. Lullman, H., R. Lullman-Rauch, and O. Wasserman. 1978. Lipidosis induced by amphilic cationic drugs. Biochem. Pharmacol. 27: 1103-1108.

43. Hughes, D.E., C.A. Schneider, and L. Simpson. 1982. Isolation and characterization of drug resistant mutants of Crithidia fasiculata. J. Parasitol. 68: 642-649.

44. North, M.J. 1982. Comparative biochemistry of the proteinases of eucaryotic microorganisms. Micro. Rev. 46: 308-340.

45. Coombs, G.H. and J. Baxter. 1984. Inhibition of Leishmania amastigote growth by antipain and leupeptin. Ann. trop. Med. Parasitol. 78: 21-24.

46. de Gee, A.L.W., P.P. McCann, and J.M. Mansfield. 1983. Role of antibody in the elimination of trypanosomes after D,L-difluoromethylornithine treatment. J. Parasitol. 69: 818-822.

47. Afchain, D., P. Desjeux, C. La Fuente, D. LeRay, J.Y. Cesbran, G.L. Heyrick, and A. Capron. 1983. Specific IgE antibodies to Leishmania braziliensis in patients with mucocutaneous leishmaniasis. Ann. Immunol. 134C: 311-319.

48. Looker, D.L., R.L. Berens, and J.J. Marr. 1983. Purine metabolism in Leishmania donovani amastigotes and promastigotes. Mol. Biochem. Parasitol. 9: 15-28 (Mini-Review).

49. Walton, B.C., J. Harper, 3rd, and R.A. Neal. 1983. Effectiveness of allopurinol against Leishmania braziliensis panamensis in Aotus trivirgatus. Amer. J. trop. Med. Hyg. 32: 46-50.

50. Avila, J.L., A. Avila, and H. Mouzon. 1984. Differences in allopurinol and 4-aminopyrozalo (3,4-d) pyrimidine metabolism in drug sensitive and insensitive strains of Trypanosoma cruzi. Mol. Biochem. Parasitol. 11: 51-60.

51. Spector, T., T.E. Jones, S.W. LaFon, D.J. Nelson, R.L. Berens, and J.J. Marr. 1984. Monophosphates of Formycin B and Allopurinol Riboside. Interactions with leishmanial and mammalian succino AMP synthetase and GMP reductase. Biochem. Pharmacol. 33: 1611-1617.

52. Urcuyo, F.G. and N. Zaias. 1983. Oral ketoconazole in the treatment of leishmaniasis. Int. J. Dermatol. 21: 414-416.

53. Weinrauch, L., R. Livshin, Z. Even-Paz, and J. El-On. 1983. Efficacy of ketoconazole in cutaneous leishmaniasis. Arch. Dermatol. Res. 275: 353-354.

54. Raether, W. and H. Seidenath. 1984. Ketoconazole and other patent antimycotic azoles exhibit pronounced activity against Trypansosoma curzi, Plasmodium berghi, and Entamoeba histolytica in vivo. Z. Parasitenkund 70: 135-138.

55. Van den Bossche, H., G. Willemsens, W.F. Lawers, and J.M. Van Cutsem. 1980. In vitro and in vivo effects of the antimycotic drug ketoconazole on sterol synthesis. Antimicrob. Agents Chemother. 17: 922-928.

56. Good, L.J., G.G. Holz, Jr., and D.H. Black. 1984. Sterols of Leishmania species. Implications for biosynthesis. Mol. Biochem. Parasitol. 19: 161-170.

57. Berman, J.D. 1981. Activity of imidazoles against Leishmania tropica in human macrophage cultures. Amer. J. trop. Med. Hyg. 30: 566-569.

58. Carson, P.E., et al.. 1981. Toxicology of the 8-aminoquinolines and genetic factors associated with their toxicity in man. Bull. World Health. Org. 59: 427-437.

59. Kinnamon, K.E. et al.. 1978. The antileishmanial activity of lepidines. Am. J. trop. Med. Hyg. 27: 751-757.

60. Berman, J.D. and L.S. Lee. 1983. Activity of 8-aminoquinolines against Leishmania tropica within human macrophages in vitro. Amer. trop. Med. Hyg. 32: 753-759.

61. Beveridge, E., I.C. Caldwell, V.S. Latter, R.A. Neal, V. Udall, and M.W. Waldron. 1980. The activity against Trypanosoma cruzi and cutaneous leishmaniasis and toxicity of moxipraquine (349C59). Trans. R. Soc. trop. Med. Hyg. 74: 43-51.

62. Stauber, L.A., E.M. Franchino, and J. Grun. 1958. An eight day method for screening compounds against Leishmania donovani in the golden hamster. J. Protozool. 5: 269-273.

63. Yau-jia, L. 1982. A review of Kala-azar in China from 1949 to 1959. Trans. R. Soc. trop. Med. Hyg. 76: 531-537.

64. Neal, R.A. 1976. Effect of sodium stibogluconate and pyrimethamine on mouse infections with Leishmania mexicana. Ann. trop. Med. Parasitol. 70:161.

65. Goodwin, L.G. 1945. The chemotherapy of experimental leishmaniasis. II. A dose response curve for the activity of sodium stibogluconate. Trans. R. Soc. trop. Med. Hyg. 34: 133-141.

66. Meshnick, S.M. and J.W. Eaton. 1981. Leishmanial superoxide dismutase: A possible target for chemotherapy. Biochem. Biophys. Res. Commun. 128:970-976.

67. McCann, P.P., C.J. Bacchi, H.C. Nathan, and A. Sjoerdsma. 1983. Difluoromethylornithine and the rational development of polyamine antagonists for the cure of protozoan infection. In: Mechanisms of Drug Action, T.P. Singer and R.N. Ondarza, eds., Academic Press, New York pp. 159-174.

68. Arrick, B.A., O.W. Griffith, and A. Cerami. 1981. Inhibition of glutathione synthesis as a chemotherapeutic strategy for trypanosomes. J. exp. Med. 153: 720-725.

69. Fairlamb, A. 1984. "Trypanothione": A novel cofactor for glutathione reductase in trypanosomes leishmania. Mol. Biochem. Parasitol. 13: (in press).

70. Desjeaux, P., F. Santoro, D. Afchain, M. Loyens, and A. Capron. 1980. Circulating immune complexes and anti-IgG antibodies in mucocutaneous leishmaniasis. Amer. J. trop.

Med. Hyg. 29: 195-198.

71. Ridley, D.S., P.D. Marsden, C.C. Cuba, and A.C. Barreto. 1980. A histological classification of mucocutaneous leishmaniasis in Brazil and its clinical evaluation. Trans. R. Soc. trop. Med. Hyg. 74: 508-514.

TABLE 1A

LEISHMANIASIS

Comparison of the suppressive effect of Pentostam and various compounds on Leishmania mexicana mexicana

Exp. No. I, II (1-3) Date 3/20 3/27 (4-8) Route 1 (9) Regimen 4 (10) Type Test A, PCP (11) Animal 3 (12) *Strain 3a (13)

1. SC 4. IM
2. IP 7. GAVAGE

1. 2X4D
2. 1 x 5D
3. 1 x 10D
4. 1 x 15D

1. HAMSTER
2. DOG
3. Mouse

1. KHARTOUM
2. brasiliensis
3. mexicana

COMPOUND NO.	MG/KG/DA	ANIMALS TOT	EXP	TOX	Mean Necropsy Weight	Mean Lesion Size (mm^2) (Weeks Post Treat.)		% SUPPRESSION Necropsy		SIG. 1. YES 2. NO	Cultures (+/ Total)
					(mg)	0	5	Original	Control		
BJ58563	660	6	3	3	29 ± 4	6.7	1.2	82	88	1	0/3
	330	6	6	0	62 ± 16	7.0	5.3	24	48	1	1/3
	165	6	5	0	54 ± 29	6.3	4.4	30	57	1	2/2
	82.5	6	6	0	149±52	6.6	7.3	0	28	1	2/2
	41.2	6	6	0	353±83	6.5	9.6	0	06	2	2/2
	20.6	4	4	0	389±129	6.7	10.5	0	0		2/2
Saline		6	6	0	395±56	6.7	10.2	0			2/2
Control		1	1	0							
BJ58563	330	6	6	0	63 ±22	7.5	4.7	37	56	1	
	247	6	6	0	68 ±43	8.0	6.0	25	44	1	
	165	6	6	0	122±65	8.0	6.8	15	36	1	
	82.5	6	6	0	186±38	7.7	8.4	0	22	1	
	41.2	6	6	0	318±42	8.3	9.8	0	08	2	
	20.6	2	2	0	318±65	7.8	10.3	0	04	2	
Saline		6	6	0	422±96	7.9	10.7	0			

TABLE 1B

LEISHMANIASIS

Comparison of the suppressive effect of Pentostam and various compounds on Leishmania mexicana mexicana

Exp. No. III (1-3) Date 4/03/84 (4-8) Route 1 (9) Regimen 4 (10) Type Test S. P (11) Animal 3 (12) *Strain 3a (13)

1. SC 4. IM
2. IP 7. GAVAGE

1. 2X4D
2. 1 x 5D
3. 1 x 10D
4. 1 x 15D

1. HAMSTER
2. DOG
3. Mouse

1. KHARTOUM
2. brasiliensis
3. mexicana

COMPOUND NO.	MG/KG/DA	ANIMALS TOT	EXP	TOX	Mean Lesion Necropsy Wt.	Mean Lesion Size (mm^2) (Weeks post Treat.)		% SUPPRESSION Necropsy		SIG. 1. YES 2. NO	Amastigotes (Mean± SD/mg lesion)
					(mg)	0	5	Orig.	Cont.		
BJ 58563	330	6	6	0	75 ±25	6.8	2.6	62	76	1	12 ±29
	247.5	6	6	0	79 ±30	5.9	2.3	61	78	1	108 ±131
	165	6	6	0	95 ±32	6.2	5.1	18	52	1	361 ±310
	82.5	6	6	0	207±72	6.3	7.8	0	26	1	2373± 2079
	41.2	6	6	0	305±101	5.8	9.2	0	13	1	2854± 1559
	20.6	4	4	0	263±48	6.0	9.0	0	15	1	3614± 2187
Saline		5	5	0	414±98	7.1	10.6				3854± 940
Normal		2	2	0	0						

TABLE 2

EFFECT OF CELL TYPE UPON EFFECTIVE DOSE OF PENTOSTAM TO SUPPRESS LEISHMANIASIS IN BALB/cByJ MICE

LEISHMANIA SUBSPECIES	[α]CELL TYPE	[β]ED 90		ED 50	
		PCP	A	PCP	A
MEXICANA MEXICANA		1090	521	290	150
BRAZILIENSIS GUYANENSIS		90	25	21	07
DONOVANI ARCHIBALDI		61	11	6	1

[α]PCP = PRIMARY CULTURE PROMASTIGOTE, A = AMASTIGOTE

[β]EFFECTIVE DOSE (ED) = MG/KG/DAY

TABLE 3A

LEISHMANIASIS

Comparison of the suppressive effect of Pentostam and various compounds on Leishmania braziliensis guyanensis

Exp. No. I, II (1-3) Date _____ (4-8) Route 1 (9) Regimen 4 (10) Type Test A, PCP (11) Animal 3 (12) *Strain 2R (13)

1. SC 4. IM 2. IP 7. GAVAGE
1. 2X4D 2. 1 x 5D 3. 1 x 10D 4. 1 x 15D
1. HAMSTER 2. DOG 3. Mouse
1. KHARTOUM 2. brasiliensis 3. mexicana

COMPOUND NO.	MG/KG/DA	ANIMALS TOT	ANIMALS EXP	ANIMALS TOX	Mean Lesion Necropsy Wt (mg)	Mean Lesion Size (mm^2) (Weeks Post Treat.) 0	Mean Lesion Size (mm^2) (Weeks Post Treat.) 5	% SUPPRESSION Necropsy Orig.	% SUPPRESSION Necropsy Cont.	SIG. 1. YES 2. NO	Culture (+/ Total)
BJ 58563	400	6	6	0	0.09	7.0	0.0	100	100	1	0/2
	330	6	6	0	0.10	8.0	0.0	100	100	1	0/2
	250	6	6	0	0.11	8.2	0.0	100	100	1	0/2
	160	6	6	0	0.09	6.9	0.0	100	100	1	2/2
	80	6	6	0	0.12	7.3	0.92	87.4	87.2	1	2/2
	40	6	6	0	0.11	6.4	0.33	95.0	95.4	1	2/2
Control +	0	4	4	0	0.27	7.8	7.20	8	1		2/2
BJ58563	330	6	6	0	0.10	6.2	0.00	100	100	1	0/2
	283	6	6	0	0.44	6.3	0.00	100	100	1	0/2
	182	6	6	0	0.11	6.8	0.00	100	100	1	0/2
	160	6	6	0	0.11	6.1	0.60	99	92	1	1/2
	80	6	6	0	0.11	6.5	0.90	86	88	1	2/2
	40	6	6	0	0.14	6.7	2.10	69	71	1	2/2
Control +	0	6	6	0	0.26	6.7	7.90	0			2/2

TABLE 3B

LEISHMANIASIS

Comparison of the suppressive effect of Pentostam and various compounds on Leishmania braziliensis guyanensis

Exp. No. III (1-3) Date 7/19/84 (4-8) Route 1 (9) Regimen 4 (10) Type Test S_2P (11) Animal 3 (12) *Strain 2R (13)

1. SC 4. IM 2. IP 7. GAVAGE
1. 2X4D 2. 1 x 5D 3. 1 x 10D 4. 1 x 15D
1. HAMSTER 2. DOG 3. Mouse
1. KHARTOUM 2. brasiliensis 3. mexicana

COMPOUND NO.	MG/KG/DA	ANIMALS TOT	ANIMALS EXP	ANIMALS TOX	Mean Lesion Necropsy Wt (mg)	Mean Lesion Size (mm^2) (Weeks Post Trest.) 0	Mean Lesion Size (mm^2) (Weeks Post Trest.) 5	% SUPPRESSION Necropsy Orig.	% SUPPRESSION Necropsy Cont.	SIG. 1. YES 2. NO	Culture (+/ Total)
BJ 58563	330	5	5	0	0.06	6.0	0.2	97	97	1	0/2
	160	6	6	0	0.06	6.5	0.0	100	100	1	1/2
	40	6	6	0	0.06	5.5	0.2	96	97	1	2/2
	10	7	7	0	0.08	6.0	4.5	25	36	1	2/2
	2.5	6	6	0	0.09	5.5	4.7	15	33	1	2/2
Control	0	6	6	0	0.21	7.0	7.4	0			2/2

TABLE 4A

LEISHMANIASIS

Comparison of the suppressive effect of Pentostam & various compounds on Leishmania donovani Khartoum

Exp. No. I (1-3) Date 7/30/84 (4-8) Route 7 (9) Regimen 2 (10) Type Test ____ (11) Animal 3 (12) Strain 1 (13)

1. SC 4. IM
2. IP 7. GAVAGE

1. 2X4D 4. 1x10 D
2. 1x5 D 5. 1x14 D
3. 1x6 D

1. HAMSTER
2. DOG
3. MOUSE

1. KHARTOUM
2. SUDAN
3. CHAGASI

Code 84	COMPOUND NO.	MG/KG/DA	ANIMALS TOT	ANIMALS EXP	ANIMALS TOX	% WT. CHANGE	MEAN # PARASITES/LIVER	% SUPPRESSION Necropsy	SIG. 1. YES 2 NO	Cultures (+/Total)
A	BD 99087	64	5	3	1	+ 1	52± 6	93	1	2/2
		32	5	4	0	- 3	73 ±13	89	1	2/2
		16	6	5	0	0	52 ±18	92	1	2/2
	BG11417	32	5	0	5	-	-	-	1	2/2
		16	6	2	0	- 2	178 ±101	74	1	2/2
		08	5	2	0	+ 4	182 ±60	88	1	2/2
C	BH73903	16	6	3	1	+ 1	92 ±45	86	1	2/2
		08	6	3	0	0	71 ±29	90	1	2/2
		04	6	3	0	+ 3	63 ±16	91	1	2/2
D	BH57098	24	5	5	0	- 5	42 ±14	94	1	2/2
		12	6	5	0	+ 2	73 ±15	89	1	2/2
		06	6	3	0	- 1	49 ±11	93	1	2/2
E	BE20578	44	5	3	1	- 8	153 ±57	77	1	2/2
		26	5	3	0	- 8	132 ±14	81	1	2/2
		11	6	3	0	- 2	81 ±17	88	1	2/2
F	BH84022	48	5	3	2	- 4	163 ±85	76	1	2/2
		24	6	3	0	+ 2	111 ±21	84	1	2/2
		12	6	3	0	- 2	73 ±26	98	1	2/2

TABLE 4B

LEISHMANIASIS

Comparison of the suppressive effect of Pentostam & various compounds on Leishmania donovani Khartoum

Exp. No. I (1-3) Date 7/30/84 (4-8) Route 7 (9) Regimen 2 (10) Type Test ____ (11) Animal 3 (12) Strain 1 (13)

1. SC 4. IM
2. IP 7. GAVAGE

1. 2X4D 4. 1x10 D
2. 1x5 D 5. 1x14 D
3. 1x6 D

1. HAMSTER
2. DOG
3. MOUSE

1. KHARTOUM
2. SUDAN
3. CHAGASI

CODE	COMPOUND NO.	MG/KG/DA	ANIMALS TOT	ANIMALS EXP	ANIMALS TOX	% WT. CHANGE	MEAN # PARASITES/LIVER	% SUPPRESSION Necropsy	SIG. 1. YES 2 NO	Cultures (+/ Total)
G	BG56256	16	5	1	4	- 20	110	84	1	2/2
		08	6	2	1	+ 2	116 ±69	83	1	2/2
		04	6	3	0	+ 4	160 ±80	76	1	2/2
H	BE66930	40	3	3	0					
		20	3	2	0					
		10	4	4	0					
I	BG56738	16	3	0	3					
		08	3	3	0					
		04	4	2	1					
J	BK 63407	12	3	0	3					
		06	3	0	3					
		03	4	0	4					
	BJ 58563	50	6	6	0	+ 6		87	1	2/2
	Control In	H_2O	6	6	0	+ 6	680 ±	0		2/2
	Control Un	H_2O	4	4	0	- 1				

TABLE 5

Toxicity of WRAIR 1984 Compounds in BALB/cByJ Mice

Code Drug	*Experimental JA/JK			WRAIR Anti-Malarial Data		
	LD50	MTD	LD100	Toxic	Active	Cured
A - BD 99087	256	320	384	640	160	
B - BG 11417	120	80	160	320	160 80 40	
C - BH 73903	108	80	180	**	--	
D - BH 57098	144	120	320	**	--	
E - BE 20578	240	220	280	-	160,80	320
F - BH 84022	240	200	260	**	--	
G - BG 56256	60	40	80	40	--	
H - BE 66930	256	200	320	640	80	
I - BG 56738	60	40	80	160	80,40	
J - BK 63407	?	?	15	**	--	

*Total Dose - mg/kg over 5 days orally

**Toxicity data unavailable from WRAIR

TABLE 6

EFFECT OF 1984 EXPERIMENTAL WRAIR DRUGS UPON LESIONS OF LEISHMANIA MEXICANA MEXICANA INFECTED BALB/CBYJ MICE

DRUG[α]	DOSE[β] MG/KG/DAY	PERCENT LESION SIZE ORIGINAL	PERCENT LESION SIZE CONTROL	SUPPRESSION % CONTROL	PENTOSTAM INDEX
A	21.3	130	75	25	5.2
B	5.3	140	92	8	8.2
C	5.3	123	97	3	5.6
D	8.0	131	89	11	4.3
E	14.7	147	103	0	-
F	5.3	133	94	6	6.0
G	1.3	132	75	25	84.0
H	4.8	128	81	19	17.0
PENTOSTAM	160.0	87	67	33	
SALINE		138	100	0	

[α]DRUG BY GAVAGE ONCE DAILY, 5 DAYS/WEEK X 3 WEEKS AT MAXIMUM TOLERATED DOSE.

[β]PENTOSTAM SUBCUTANEOUSLY DAILY, AS ABOVE AT EFFECTIVE DOSE

N = 6 MICE/GROUP

TABLE 7

EFFECT OF DFMO + PENTOSTAM UPON LESIONS OF *LEISHMANIA MEXICANA MEXICANA* INFECTED BALB/cByJ MICE

PENTOSTAM[α] MG/KG/DAY	DFMO[β] %	PERCENT LESION SIZE ORIGINAL	PERCENT LESION SIZE CONTROL	SUPPRESSION % CONTROL
160	3	96	89	11
	2	99	87	13
	1	100	88	12
	0.5	100	84	16
80	3	100	100	0
	2	100	91	9
	1	100	90	10
	0.5	100	98	2
40	3	100	98	2
	2	100	91	9
	1	100	100	0
	0.5	100	100	0

[α] DFMO IN DRINKING WATER 3 DAYS PRIOR TO PENTOSTAM; DAILY FOR 3 WEEKS.

[β] PENTOSTAM SUBCUTANEOUSLY 5 DAYS/WEEK x 3 WEEKS
N = 5 MICE/GROUP

TABLE 8

PERCENT SUPPRESSION OF *LEISHMANIA BRAZILIENSIS GUYANENSIS* LESIONS BY DFMO + PENTOSTAM[Ψ]

PENTOSTAM	PERCENT DFMO 0	0.5	1.0	2.0	3.0	4.0	5.0
0	0	0	15	35*	3	10	46*
6.25	0	21	14	13	32	10	37
12.50	0	0	3	14	0	0	13
25.00	5	0	5	0	0	0	12
50.00	0	0	5	25*	0	0	34
BLEOMYCIN	25*	-	0	-	0	-	-

*SIGNIFICANT DIFFERENCE ALSO % ORIGINAL LESION SIZE

[Ψ] ONE WEEK POST TREATMENT. N = 6 MICE/GROUP

Table 9

EFFECT OF MEDIA ON THE TRANSFORMATION OF L. B. Brasiliensis M 2904

		Days in Culture (Cells x 10^6)						
Base	Overlay	1	2	3	4	5	6	
Blood Agar	cSDM	0.13	0.13	0.75^{T}	0.50	21.5	25.0	
"	SDM	0.63	0.00	0.13^{T}	1.13^{M}	12.0	3.0	
"	HBSS	0.63	3.00	0.90^{T}	1.75^{M}	15.8	24.5	Hamster
Plain Agar	cSDM	0.38	0.25	0.25^{M}	1.75	3.50	23.0	Origin
"	SDM	0.50	0.00	0.50	0.00	0.00	0.00	
"	HBSS	0.25	0.00	0.13	0.00	0.00	0.00	
No Agar	cSDM	0.13	0.63	0.25^{T}	2.00	2.50	4.00	
Blood Agar	cSDM	0.13	3.63	4.30^{T}	17.5^{VM}	54.5	56.5	
"	SDM	1.00	2.30	3.30^{T}	11.5^{VM}	23.3	10.0	
"	HBSS	1.00	0.00^{T}	2.13^{S}	9.80^{VM}	40.0	54.5	Mouse
Plain Agar	cSDM	0.13	1.50^{T}	2.13^{E}	7.30	15.5	56.0	Origin
"	SDM	0.50	0.00	0.13	0.00	0.00	0.00	
"	HBSS	0.63^{T}	0.00	0.13	0.00	0.00	0.00	
No Agar	cSDM	0.50	0.13	3.40^{E}	9.80^{VM}	9.30	9.30	

Conclusions: Mouse better than hamster
Agar needed for transformation
Serum needed to sustain growth

T= transformed
M= motile
VM= very motile
S= small
E= elongated

Table 10 A

Leishmania brasiliensis brasiliensis: Infection in mice with parasites from mouse origin versus hamster origin

	Wks:	1	2	3	4	5	6	7	8	10
M→M Sch	Mean	1.0	1.0	4.0	1.0	0.0	1.0	1.0	4.7	4.7
	s.d.	-	-	-	-	-	-	-	1.1	0.3
	%pos.	40	40	40	20	0	20	20	40	40
M→M BA	Mean	3.1	3.0	3.1	2.5	3.0	2.8	2.0	3.7	4.2
	s.d.	0.4	1.2	1.4	2.1	2.8	1.7	2.0	2.7	3.0
	%pos.	100	100	80	40	40	80	80	60	60
H→M Sch	Mean	3.6	1.0	2.2	1.0	0.0	0.0	1.0	4.8	1.0
	S.D.	0.5	--	1.8	-	-	--	-	0.6	--
	%pos.									
H→M BA	Mean	1.2	3.9	4.0	2.7	2.5	1.5	2.5	3.6	5.0
	s.d.	0.5	0.6	0.5	0.3	-	0.4	1.5	1.8	--
	%pos	80	80	60	60	60	60	60	80	20

Table 10B

Leishmania brasiliensis brasiliensis: Infection in Hamsters with parasites from mouse origin vs. hamster origin.

Hamster to Hamster:

Blood Agar: +4, +3, +2
Schneiders: +2, +2

Mouse to Hamster:

Blood Agar: +3, +2, +1
Schneiders: +3, +2, +2

0= no change
+1=hair loss
+2=slight enlargement
+3=enlargement
+4=very enlarged

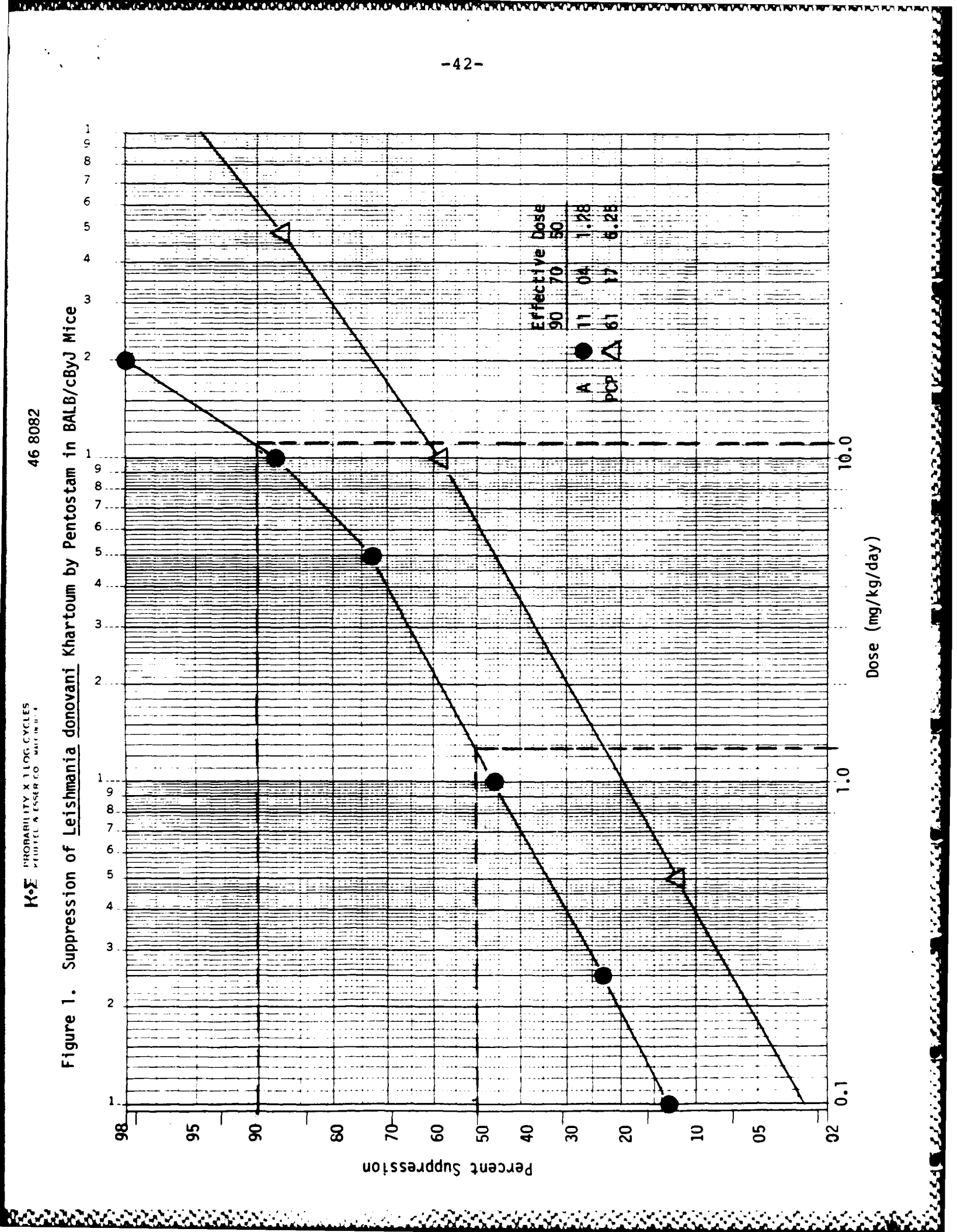


Figure 1. Suppression of Leishmania donovani Khartoum by Pentostam in BALB/cByJ Mice

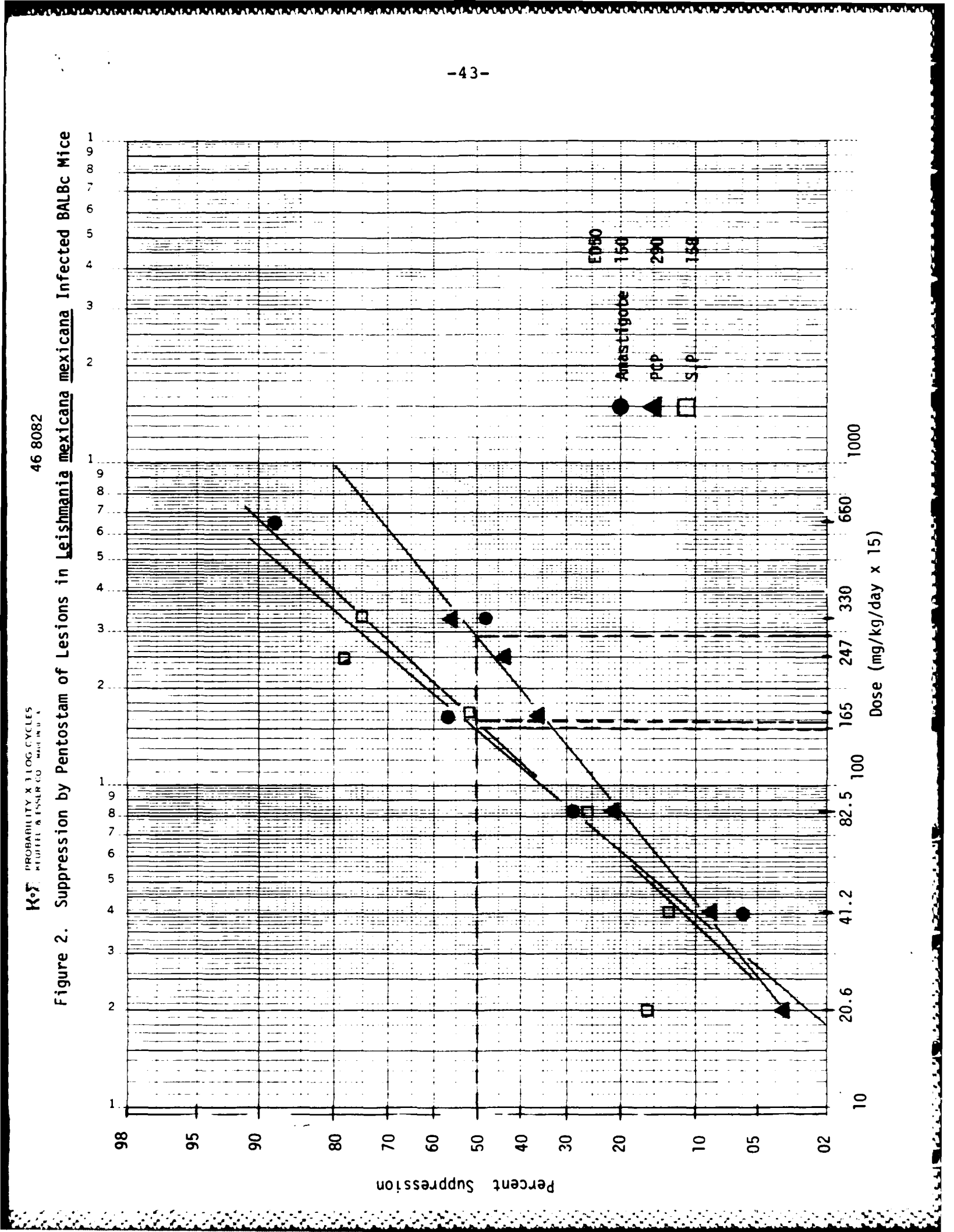


Figure 2. Suppression by Pentostam of Lesions in Leishmania mexicana mexicana Infected BALBc Mice

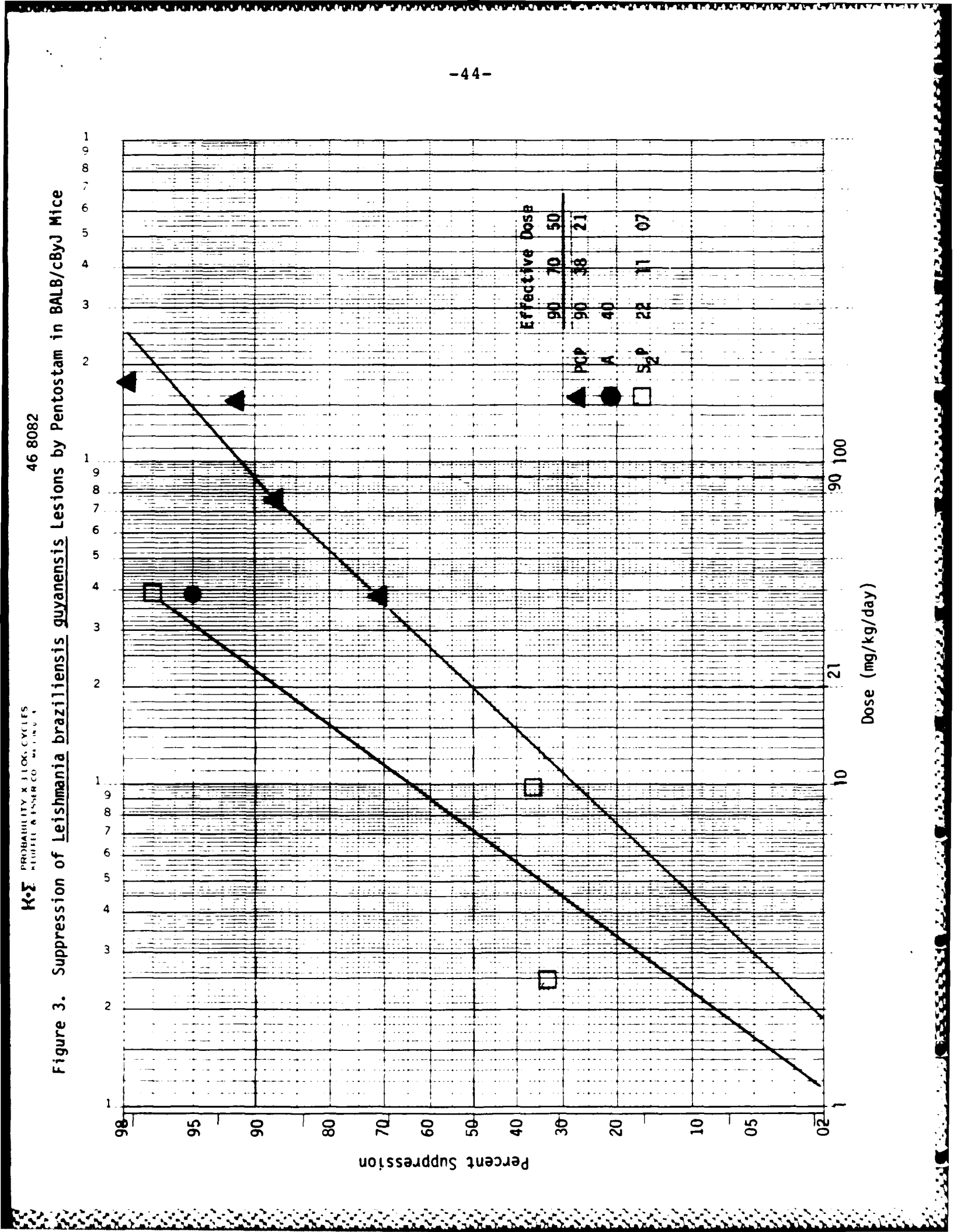


Figure 3. Suppression of Leishmania braziliensis guyanensis Lesions by Pentostam in BALB/cByJ Mice

VII. Distribution List

A. Commander
U.S. Army Medical Research and Development Command — 2
ATTN: SGRD-RMS
Fort Detrick
Frederick, Maryland 21701

B. Howard E. Noyes, Director — 1
Office of Research Management
SGRD-UWZ-C
Walter Reed Army Institute of Research
Washington, D.C. 20307

C. Major James Lovelace, COTR — 1
Walter Reed Army Institute of Research
Department of Parasitology
Division of Experimental Therapeutics
Washington, D.C. 20012

D. Ann W. Stanford — 1
Research Administrator
Office of Research and Sponsored Programs
Cornell University Medical College
1300 York Avenue
New York City 10021

E. Thomas C. Jones, M.D. — 1
Chief, Division of International Medicine A-431
Department of Medicine
Cornell University Medical College
1300 York Avenue
New York City 10021

Merrell Dow Research Institute

MERRELL DOW PHARMACEUTICALS INC.
2110 E. Galbraith Road
Cincinnati, Ohio 45215, U.S.A.

Phone 513-948-9111
Telex 214320 Merrell Read

August 30, 1984

Dr. Jan S. Keithly
Cornell University Medical College
1300 York Ave.
New York, NY 10021

Dear Dr. Keithly:

Enclosed is a 300.0 gram sample of alpha-difluoromethylornithine (MDL 71,782 A) for use in the antileishmania studies in mice as outlined in your Statement of Investigator form.

For your personal use we are enclosing three pertinent reprints which may be helpful in your studies.

Also enclosed is a summary sheet on the chemistry and pharmacology of MDL 71,782 A.

We look forward to a report of the results of these studies when they are completed.

Sincerely,

W.J. Hudak, Ph.D.
Manager, Research Information

WJH:cs

Enc.

For Acknowledgements: Merrell Dow Research Institute

Merrell Dow Research Institute

MERRELL DOW PHARMACEUTICALS INC.
2110 E. Galbraith Road
Cincinnati, Ohio 45215, U.S.A.

Phone 513-948-9111
Telex 214320 Merrell Read

April 6, 1984

Dr. Jan S. Keithly
Division of International Medicine
Department of Medicine
The New York Hospital-Cornell Medical Center
525 East 68th Street
New York, New York 10021

Dear Jan,

I was delighted to speak to you today about everything. I enclose the statement of investigator forms and some recent references you may or may not have seen.

Very best wishes,

Peter P. McCann, Ph.D.
Senior Biochemist

Associate Professor of Cell Biology
University of Cincinnati College of Medicine

PPM/bjh

(enclosures)

REFERENCES:

1) Metcalf, B.W., Bey, P., Danzin, C., Jung, M.J., Casara, P. and Vevert, J.P. Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C. 4.1.1.17) by substrate and product analogs. J. Am. Chem. Soc., 100:2551-53 (1978)

2) Danzin, C., Jung, M.J., Grove, J. and Bey, P. Effect of α-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase, on polyamine levels in rat tissues. Life Sci. 24:519-524 (1979)

3) Mamont, P.S., Duchesne, M.-C., Grove, J. and Bey, P. Antiproliferative properties of D,L-α-difluoromethylornithine in cultured cells. A consequence of the irreversible inhibition of ornithine decarboxylase. Biochem. Biophys. Res. Commun. 81:58-66 (1978)

4) Luk, G.D., Goodwin, G., Marton, L.J. and Baylin, S.B. Polyamines are necessary for the survival of human small-cell lung carcinoma in culture. Proc. Natl. Acad. Sci. USA 78:2355-2358 (1981)

5) Prakash, N.J., Schechter, P.J., Mamont, P.S., Grove, J., Koch-Weser, J. and Sjoerdsma, A. Inhibition of EMT-6 tumor growth by interference with polyamine biosynthesis; effects of α-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase. Life Sci. 26:181-194 (1980)

6)* Sjoerdsma, A. Suicide enzyme inhibitors as potential drugs. Clin. Pharmacol. Ther. 30:3-22 (1981)

7)* Pegg, A.E. and McCann, P.P. Polyamine metabolism and function: A brief review. Am. J. Physiol. 243:C212-C221 (1982)

8) Fozard, J.R., Part, M-L., Prakash, N.J., Grove, J., Schechter, P.J., Sjoerdsma, A. and Koch-Weser, J. L-Ornithine decarboxylase: an essential role in early mammalian embryogenesis. Science 208:505-508 (1980)

9) Bacchi, C.J., Nathan, H.C., Hutner, S.H., McCann, P.P. and Sjoerdsma, A. Polyamine metabolism: a potential therapeutic target in trypanosomes. Science 210:332-334 (1980)

10)* Sjoerdsma, A. and Schechter, P.J. Chemotherapeutic implications of polyamine biosynthesi inhibition. Clin. Pharmacol. Ther. (1983) in press

* Reprints-preprints available from Merrell Dow.

525 EAST 68th STREET NEW YORK N Y 10021

THE NEW YORK HOSPITAL-CORNELL MEDICAL CENTER

DEPARTMENT OF PEDIATRICS
DIVISION OF PEDIATRIC HEMATOLOGY-ONCOLOGY

April 10, 1984

Janet S. Keithly, Ph.D.
Department of Medicine
Division of International Medicine
Cornell University Medical Center
New York, NY 10021

Dear Dr. Keithly:

Pursuant to our recent conversations, I would be most happy to collaborate with you on studies of iron metabolism in Leishmania.

Two separate approaches seem appropriate. Given the environment in which Leishmania flourish, it is reasonable that they would secrete a siderophore in order to sequester iron necessary for growth. The capacity to produce such a siderophore could indeed be a major factor in determining virulence. Isolation and identification of this putative virulence factor should aid in the development of a highly sensitive and selective model for screening antileishmanial agents. I will undertake the chemical aspects of such a characterization.

Apart from contributing to the development of an appropriate screening system, identification of the abovementioned siderophore should provide clues for designing chemotherapeutic agents which interfere with iron utilization by these organisms. I will provide you with a variety of iron-chelating agents to screen as potential drugs. These could be screened in vitro as well as in the model that you are developing. I am also willing to synthesize new chelators as a result of identifying the leishmanial siderophore and/or the results of screening studies.

Sincerely,

Robert W. Grady, Ph.D.
Assistant Professor
Department of Pediatrics/
Pharmacology

THE DIVISION OF PEDIATRIC HEMATOLOGY/ONCOLOGY
IS SUPPORTED BY THE CHILDREN'S BLOOD FOUNDATION

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person. [PII Redacted]

NAME	TITLE	
Robert W. Grady, Ph.D.	Assistant Professor, Dept. of Pediatrics/Pharmacology	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (if any) (Circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
Brown University	B.S.	1963	Organic Chemistry
University of Minnesota	Ph.D.	1971	Biochemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Positions Held:

1979-present	Assistant Professor, Cornell University Medical Center
1979-1983	Adjunct Assistant Professor, The Rockefeller University
1975-1979	Assistant Professor, The Rockefeller University
1972-1975	Research Associate, The Rockefeller University
1971-1972	Post-doctoral Fellow, Albert Einstein College of Medicine

Memberships:

American Chemical Society
New York Academy of Sciences
American Association for the Advancement of Science
International Society of Hematology

Publications: (Total 42: Original Articles 35; Review Articles 7)

Grady, R.W., Graziano, J.H., White, G.P., Jacobs, A. and Cerami, A.: The development of new iron-chelating drugs. II. J. Pharmacol. Exp. Ther., 205:757-765, 1978.

Meshnick, S.R., Blobstein, S.H., Grady, R.W. and Cerami, A.: An approach to the development of new drugs for African trypanosomiasis. J. Exp. Med., 148:569-579, 1978.

Blobstein, S.H., Grady, R.W., Meshnick, S.R. and Cerami, A.: Hydroxamic acid oxidation: Pharmacological considerations. Biochem. Pharmacol., 27:2939-2945, 1978.

Meshnick, S.R., Grady, R.W., Blobstein, S.H. and Cerami, A.: Porphyrin induced lysis of Trypanosoma brucei. A role for zinc. J. Pharmacol. Exp. Ther., 207:1041-1050, 1978.

Grady, R.W., Peterson, C.M., Jones, R.L., Graziano, J.H., Bhargava, K.K., Berdoukas, V.A., Kokkini, G., Loukopoulis, D. and Cerami, A.: Rhodotorulic acid - Investigation of its potential as an iron-chelating drug. J. Pharmacol. Exp. Ther., 209:342-348, 1979.

Jones, R.L., Peterson, C.M., Grady, R.W. and Cerami, A.: Low molecular weight iron-binding factor from mammalian tissue that potentiates bacterial growth. J. Exp. Med. 151:418-428, 1980.

Bhargava, K.K., Grady, R.W. and Cerami, A.: New compounds: N^1,N^8-Bis(2,3-dihydroxybenzoyl)spermidine and analogues as potential iron-chelating drugs. J. Pharm. Sci. 69:986-989, 1980.

Clarkson, A.B. Jr., Grady, R.W., Grossman, S.A., McCallum, R.J. and Brohn, F.H.: Trypanosoma brucei brucei: A systemic screening for alternatives to the salicylhydroxamic acid/glycerol combination. Mol. Biochem. Parasitol. 3:271-291, 1981.

Grady, R.W. and Jacobs, A.: The screening of potential iron chelating drugs. in "Development of Iron Chelators for Clinical Use." A.E. Martell, W.F. Anderson and D.G. Badman (eds.), Elsevier/North Holland, Inc., pp. 133-164, 1981.

Clarkson, A.B. Jr. and Grady, R.W.: The use of hydroxamic acids to block electron transport in pathogenic African trypanosomes. in "Proceedings of the First International Symposium on the Biology and Chemistry of Hydroxamic Acids." H. Kehl (ed.), S. Kagen, Berlin, pp. 130-139, 1982.

Ullrich, P.C., Grady, R.W. and Cerami, A.: The trypanocidal activity of various aromatic bisguanylhydrazones in vivo. Pharmacol. Drug Develop. Res. 2:219-228, 1982.

Hershko, C., Grady, R.W., Link, G., Sarel, S. and Avramovici-Grisaru, S.: In vivo evaluation of the new iron-chelating drugs. in "Fifth International Conference on Proteins of Iron Storage and Transport." P. Saltman and J. Hegenaur (eds.), Elsevier/North Holland, pp. 627-648, 1982.

de Sousa, M., Martins da Silva, B., Dorner, M, Munn, G., Nishiya, K., Grady, R.W. and Silverstone, A.: Iron and the lymphomyeloid system: Rationale for considering iron as a target of immune surveillance. in "Fifth International Conference on Proteins of Iron Storage and Transport." P. Saltman and J. Hegenauer (eds.), Elsevier/North Holland, pp. 687-698, 1982.

Hershko, C., Grady, R.W. and Link, G.: Evaluation of iron chelating agents in an in vivo system: Potential usefulness of EHPG, a powerful iron chelating drug. Br. J. Haematol. 51:251-260, 1982.

Jones, R.L. and Grady, R.W.: Siderophores as antibiotics. Eur. J. Microbiol. 2:373-375, 1983.

Jones, R.L., Grady, R.W. and Peterson, C.M.: Hemochromatosis: Pathogenesis, diagnosis and treatment. Contemp. Issues Clin. Nutr. 5:167-201, 1983.

Blobstein, S.H., Grady, R.W., Meshnick, S.R., Ullrich, P.C., Cerani, A., Amirmoazzami, J. and Hodnett, E.M.: The in vitro trypanocidal activity of N-substituted p-benzoquinone imines: Assessment of biochemical structure activity relationships using the Hansch approach. J. Cell. Biochem. (in press) 1984.

Grady, R.W., Akbar, A.N., Giardina, P.J., Hilgartner, M.W. and de Sousa, M.: Abnormal proportions of lymphoid cell subsets in thalassemia major: The relative contributions of transfusion and splenectomy. N. Engl. J. Med. (submitted) 1983.

Ponka, P., Grady, R.W., Wilczynska, A. and Schulman, H.M.: Studies on iron metabolism and hemoglobin synthesis in reticulocytes using pyridoxal isonicotinoyl hydrazone and some other chelating agents. J. Pharmacol. Exp. Ther. (submitted) 1983.

1300 YORK AVENUE, NEW YORK N Y 10021

THE NEW YORK HOSPITAL-CORNELL MEDICAL CENTER

DEPARTMENT OF BIOCHEMISTRY

April 11, 1984

Dr. Janet Keithly
Department of Medicine
Cornell University Medical College
New York, NY 10021

Dear Dr. Keithly:

I am writing to formally confirm our agreement to collaborate on the following projects:

1) A study of the mechanism of action of pentostam in vivo and in vitro in protozoa such as leishmania My laboratory will prepare radiolabelled pentostam and will assist in determining the effects of glutathione depletion on pentostam toxicity.

2) A study of the use of carnitine analogs to inhibit growth and division of lei hmania My laboratory has prepared several analogs which are designed to interfere with particular steps in fatty acid oxidation.

I thank you for the opportunity to participate in these projects and look forward to beginning studies as soon as possible.

Sincerely,

Owen W. Griffith

Owen W. Griffith, Ph.D.
Associate Professor

OWG:ng

February 1984

CURRICULUM VITAE

PII Redacted

Name: Owen W. Griffith, Ph.D. [redacted]

[redacted]

Education:

B.A. (Biochemistry) 1968, University of California (Berkeley)

Ph.D. (Biochemistry) 1974, The Rockefeller University, New York City

Professional Experience:

July 1981 - Associate Professor, Department of Biochemistry, Cornell University Medical College, New York, N.Y. (Tenured)

July 1978 - Assistant Professor, Department of Biochemistry, Cornell University Medical College, New York, N.Y.

January 1978 - Instructor, Department of Biochemistry, Cornell University Medical College, New York, N.Y.

July 1974 - Postdoctoral Fellow, Department of Biochemistry, Cornell University Medical College, New York, N.Y.

Honors and Awards:

Phi Beta Kappa; Woodrow Wilson Designate; NSF Predoctoral Fellowship, 1968 - 1971; NIH Postdoctoral Fellowship, 1975 - 1977;
Andrew Mellon Teacher-Scientist, 1978-1980.
Irma T. Hirschl Fellowship 1981-1985.

Professional Societies:

Member, American Society of Biological Chemists
Member, American Chemical Society
Member, New York Academy of Sciences
Member, American Association for the Advancement of Science

Major Research Interests:

Amino Acid metabolism; carnitine and the control of fatty acid oxidation; enzyme mechanisms and synthesis of enzyme specific substrates and inhibitors; *in vivo* manipulation of metabolic pathways; biochemistry of inherited metabolic disorders.

CURRENT RESEARCH

My studies center on the elucidation of amino acid and fatty acid metabolism in the intact animal. Specifically radiolabeled substrates or substrate analogs are used to monitor the metabolic flux through individual pathways and enzyme-specific inhibitors are used to predictably manipulate key metabolic pathways *in vivo*. Present investigations concern cysteine catabolism, glutathione turnover, β-amino acid metabolism, and the role of carnitine in fatty acid oxidation; specific studies in each of these areas are outlined below.

In mammals, cysteine is catabolized by at least 8 distinct pathways. Although a multitude of intermediate products are formed, the ultimate catabolites are few (pyruvate, $SO_4^=$, taurine). The enzymology of many of the reactions involved is well defined *in vitro*, but little is known of the relative importance of the various pathways *in vivo*. I am using radiolabeled cysteine and cysteine analogs (α-methylcysteine, β-methylcysteine) to determine the capacity of various pathways of cysteine metabolism *in vivo*; compounds radiolabeled in either C-1 or C-3 are used to determine the products ultimately formed (i.e. C-1 is metabolized to CO_2 by all of the established pathways whereas C-3 is metabolized to CO_2 only if taurine is not formed). The partitioning of cysteinesulfinate, a major cysteine catabolite, between decarboxylation and transamination pathways of metabolism was recently determined by this technique (38)[1/]. Inhibitors of cysteinesulfinate decarboxylase have been administered to animals to increase the percentage of cysteinesulfinate transaminated (38); animals treated in this way will be used to quantitatively evaluate alternative pathways of taurine formation (e.g. via coenzyme A and cysteamine). This general approach will ultimately be extended to the other pathways of cysteine metabolism with the goal of determining which reactions are primarily responsible for maintaining the tissue cysteine concentration at its normally very low level (100-200 uM).

Studies of glutathione turnover center on the use of enzyme-selective inhibitors to control glutathione synthesis (8,9,14,24,37) or catabolism (12). Inhibitors of glutathione synthesis have been shown to be therapeutic for animal trypanosomiasis, a model for African sleeping sickness (27). Glutathione depletion has been shown to increase the sensitivity of tumor cells to oxygen-mediated cytolysis

1/ References are numbered to correspond to the list of publications beginning on p. 6.

by leukocytes (35) and to prevent the synthesis of leukotriene C (29) and prostaglandin E_2 (34) in macrophages. These inhibitors have also been used to evaluate the rate and control of glutathione synthesis in various cells and tissues (9,12, 15,17,21,23,26,30,32,33). These studies are continuing and now focus on the design and synthesis of compounds with better pharmacologic properties (i.e., slower renal excretion and, in the case of γ-glutamyltranspeptidase inhibitors, lower toxicity). The role of γ-glutamyltranspeptidase in extracellular glutathione oxidation is also under investigation; the relative role of transpeptidase (19) and glutathione oxidase in vivo is presently poorly defined and in dispute. Specific inhibitors of γ-glutamyltranspeptidase are being applied to this problem.

Studies of β-amino acid metabolism focus on the catabolism of β-amino-iso-butyrate. We have purified β-amino-iso-butyrate - pyruvate transaminase from rat liver mitochondria and are investigating the substrate specificity of this enzyme with the view of establishing its role in the metabolism of other β-amino acids (e.g. β-alanine and hypotaurine). Selective inhibitors of this enzyme have been designed and are being evaluated both in vitro and in vivo. Enzyme inhibition produces a marked urinary excretion of β-amino-iso-butyrate. Since R-(-)β-amino-iso-butyrate is a product of thymine catabolism, the enzyme inhibitors may facilitate the in vivo evaluation of thymine turnover; such measurements may be important in the early detection of some forms of cancer.

Studies of carnitine metabolism were initiated only recently. Analogs of carnitine (methyl substituted derivatives as well as analogs in which the hydroxyl is replaced by SH or NH_2) have been prepared and used to evaluate the specificity of the carnitine acyltransferases. The results of these studies will form a basis for the rational design of enzyme-specific inhibitors. These investigations have two goals, (i) the development of animal models for the carnitine deficiency syndromes (inherited human disorders characterized by several muscular and systemic derangements of varying severity) and (ii) the synthesis of compounds which can control ketogenesis in the whole animal. The latter may be of use in controlling some of the complications of diabetes.

PUBLICATIONS

Griffith, O.W., "Mechanism of Carnitine Acetyltransferase", Ph.D. Thesis, The Rockefeller University (1974).

1. Van Der Werf, P., Griffith, O.W., and Meister, A.: "5-Oxo-L-Prolinase (L-Pyroglutamate Hydrolase); Purification and Catalytic Properties", Journal of Biological Chemistry, 250, 6686-6692 (1975).

2. Griffith, O.W., Van Der Werf, P., and Meister, A.: "Specificity and Mechanism of Action of 5-Oxoprolinase", in Glutathione: Metabolism and Function, (I.M. Arias and W.B. Jakoby, eds.), Krok Foundation Symposium; Santa Barbara, California. June 1-3, 1975; pp. 63-69; Raven Press (1976).

3. Griffith, O.W., and Meister, A.: "Dependence of Energy Coupling on Nucleotide Base Structure in the Reaction Catalyzed by 5-Oxo-L-Prolinase". Biochemical Biophysical Research Communications, 70, 759-765 (1976).

4. Griffith, O.W., and Meister, A.: "Selective Inhibition of γ-Glutamyl Cycle Enzymes by Substrate Analogs", Proceedings of the National Academy of Sciences, U.S., 74, 3330-3334 (1977).

5. Griffith, O.W., Larsson, A., and Meister, A.: "Inhibition of γ-Glutamylcysteine Synthetase by Cystamine; An approach to a Therapy of 5-Oxoprolinuria (Pyroglutamic Aciduria), Biochemical Biophysical Research Communications, 79, 919-925 (1977).

6. Powers, S.G., Griffith, O.W., and Meister, A.: "Inhibition of Carbamyl Phosphate Synthetase by P^1, P^5 - Di(adenosine 5')-pentaphosphate", Journal of Biological Chemistry, 252, 3558-3560 (1977).

7. Griffith, O.W., and Meister, A.: "Differential Inhibition of Glutamine and γ-Glutamylcysteine Synthetases by α-Alkyl Analogs of Methionine Sulfoximine that Induce Convulsions", Journal of Biological Chemistry, 253, 2333-2338 (1978).

8. Griffith, O.W., Anderson, M.E., and Meister, A.: "Inhibition of Glutathione Biosynthesis by Prothionine Sulfoximine (S-n-Propyl-Homocysteine Sulfoximine), A Selective Inhibitor of γ-Glutamylcysteine Synthetase", Journal of Biological Chemistry, 254, 1205-1210 (1979)

9. Griffith, O.W., Bridges, R.J., and Meister, A.: "Evidence that the γ-Glutamyl Cycle Functions In Vivo Using Intracellular Glutathione; Effects of Amino Acids and Selective Inhibition of Enzymes". Proceedings of the National Academy of Sciences, U.S. 75, 5405-5408 (1978)

10. Cooper, A.J.L. and Griffith, O.W.: "N-Hydroxyamino Acids: Irreversible Inhibitors of Pyridoxal 5'-phosphate Enzymes and Substrates of D- and L-Amino Acid Oxidases", Journal of Biological Chemistry, 254, 2748-2753 (1979).

11. Meister, A., and Griffith, O.W.: Effects of Methionine Sulfoximine Analogs on the Synthesis of Glutamine and Glutathione; Possible Chemotherapeutic Implication, Conference on Relevance in Treatment of Cancer of Amino Acid Depleting Enzymes and Antimetabolites of Such Amino Acids; May 23, 1978, National Cancer Institute, Bethesda, Maryland, Cancer Chemotherapy Reports, 63, 1115-1121 (1979).

12. Griffith, O.W., and Meister, A.: "Translocation of Intracellular Glutathione to Membrane-Bound γ-Glutamyl Transpeptidase as a Discrete Step in the γ-Glutamyl Cycle; Glutathionuria After Inhibition of Transpeptidase", Proceedings of the National Academy of Sciences, U.S., 75, 268-272 (1979).

13. Oppenheimer, L., Wellner, V.P., Griffith, O.W., and Meister, A.: "Glutathione Synthetase; Purification from Rat Kidney and Mapping of the Substrate Binding Sites", Journal of Biological Chemistry, 254, 5184-5190 (1979).

14. Griffith, O.W., and Meister, A.: "Potent and Specific Inhibition of Glutathione Synthesis by Buthionine Sulfoximine (S-n-Butyl-Homocysteine Sulfoximine)", Journal of Biological Chemistry, 254, 7558-7560 (1979).

15. Griffith, O.W., Novogrodsky, A., and Meister, A.: "Translocation of Glutathione from Lymphoid Cells That Have Markedly Different γ-Glutamyl Transpeptidase Activities", Proceedings of the National Academy of Sciences, 76, 2249-2252 (1979).

16. Griffith, O.W., and Meister, A.: "Glutathione: Interorgan Translocation, Turnover, and Metabolism", Proceedings of the National Academy of Sciences, U.S., 76, 5606-5610 (1979).

17. Griffith, O.W., Bridges, R.J., and Meister, A.: "Transport of γ-Glutamyl Amino Acids: Role of Glutathione and γ-Glutamyl Transpeptidase", Proceedings of the National Academy of Sciences, U.S., 76, 6319-6322 (1979).

18. Meister, A., Griffith, O.W., Novogrodsky, A., and Tate, S.S.: "New Aspects of Glutathione Metabolism and Translocation in Mammals", Sulfur in Biology, Ciba Foundation Symposium, 72, 135-161, Elsevier, Amsterdam (1980).

19. Griffith, O.W., and Tate, S.S.: "The Apparent Glutathione Oxidase Activity of γ-Glutamyl Transpeptidase: Chemical Mechanism", Journal of Biological Chemistry, 255, 5011-5014 (1980).

20. Cooper, A.J.L., Griffith, O.W., Meister, A., and Field, F.H.: "Chemical Ionization Mass Spectra of L-Methionine and L-Methionine Analogs", Biomedical Mass Spectrometry, 8, 95-98 (1981).

21. Griffith, O.W., and Meister, A.: "Excretion of Cysteine and γ-Glutamylcysteine Moieties in Human and Experimental Animal γ-Glutamyl Transpeptidase Deficiency", Proceedings of the National Academy of Sciences, U.S., 77, 3384-3387 (1980).

22. Griffith, O.W.: "Determiniation of Glutathione and Glutathione Disulfide Using Glutathione Reductase and 2-Vinylpyridine", Analytical Biochemistry 106, 207-212 (1980).

23. Bridges, R.J., Griffith, O.W., and Meister, A.: "Threo-L-γ-(β-Methyl) glutamyl-L-α-aminobutyrate, a Specific Substrate of γ-Glutamyl Cyclotransferase: In Vivo Inhibition by β-Aminoglutaryl-L-α-aminobutyrate", Journal of Biological Chemistry, 255, 10787-10792 (1980).

24. Griffith, O.W.: "Depletion of Glutathione by Inhibition of Biosynthesis", in Methods in Enzymology (Jakoby, W.B., ed.), Academic Press, New York, 77, 59-64 (1981).

25. Beamer, R.L., Griffith, O.W., Gass, J.D., Anderson, M.E. and Meister, A.: "Interaction of L- and D-3-Amino-1-chloro-2-pentanone with γ-Glutamylcysteine Synthetase", Journal of Biological Chemistry, 255, 11732-11736 (1980).

26. Griffith, O.W.: "Glutathione Turnover in Human Erythrocytes: Inhibition by Buthionine Sulfoximine and Incorporation of Glycine by Exchange", Journal of Biological Chemistry, 256, 4900-4904 (1981).

27. Arrick, B.A., Griffith, O.W., and Cerami, A.: "Inhibition of Glutathione Synthesis as a Chemotherapeutic Strategy for Trypanosomiasis", Journal of Experimental Medicine, 153, 720-725 (1981).

28. Meister, A., Tate, S.S., and Griffith, O.W.: "γ-Glutamyl Transpeptidase", in Methods in Enzymology (Jakoby W.B. ed.), Academic Press, New York, 77, 237-253 (1981).

29. Rouzer, C., Scott, W.A., Griffith, O.W., Hamill, A.L. and Cohn, Z.A.: "The Depletion of Glutathione Selectively Inhibits the Synthesis of Leukotriene C by Mouse Peritoneal Macrophages", Proceedings of the National Academy of Sciences, 78, 2532-2536 (1981).

30. Griffith, O.W., Bridges, R.J., and Meister A.: "Formation of γ-Glutamyl-cysteine In Vivo is Catalyzed by γ-Glutamyl Transpeptidase", Proceedings of the National Academy of Sciences, U.S., 78, 2777-2781 (1981).

31. Griffith, O.W., and Meister, A.: "5-Oxo-L-prolinase (L-Pyroglutamate Hydrolase): Studies of the Chemical Mechanism", Journal of Biological Chemistry, 256 , 9981-9985 (1981).

32. Griffith, O.W.: "The Role of Glutathione Turnover in the Apparent Renal Secretion of Cystine", Journal of Biological Chemistry, 256, 12263-12268 (1981).

33. Rouzer, C., Scott, W.A., Griffith, O.W., Hamill, A.L. and Cohn, Z.: "Glutathione Metabolism in Resting and Phagocytizing Peritoneal Macrophages", Journal of Biological Chemistry, 257, 2002-2008 (1982).

34. Rouzer, C., Scott, W.A., Griffith, O.W., Hamill, A.L. and Cohn, Z.A.: "Arachidonic Acid Metabolism in Glutathione Deficient Macrophages", Proceedings of the National Academy of Sciences, 79, 1621-1625 (1982).

35. Arrick, B.A., Nathan, C., Griffith, O.W., and Cohn, Z.A.: "Glutathione Depletion Sensitizes Tumor Cells to Oxidative Cytolysis", Journal of Biological Chemistry, 257, 1231-1237 (1982).

36. Griffith, O.W. and Meister A.: "Interaction of 5-Oxo-L-Prolinase with Nucleoside Triphosphates: Evidence Suggesting Substrate-Dependent Conformational Change", Journal of Biological Chemistry, 257, 4392-4397 (1982).

37. Griffith, O.W.: "Mechanism of Action, Metabolism and Toxicity of Buthionine Sulfoximine and Its Higher Homologs, Potent Inhibitors of Glutathione Biosynthesis", Journal of Biological Chemistry 257, 13704-13712 (1982).

38. Griffith, O.W.: "Cysteinesulfinate Metabolism: Altered Partitioning Between Transamination and Decarboxylation Following Administration of β-Methyleneaspartate", Journal of Biological Chemistry 258, 1591-1598 (1983).

TABLE 1

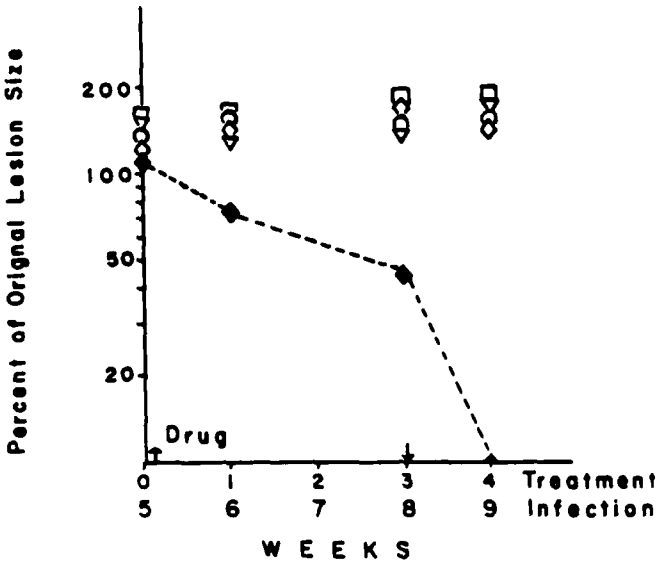
Appendix II

EFFECT OF TIOCONAZOLE CREAM UPON ULCERATED AND CLOSED LESIONS CAUSED BY Leishmania braziliensis guyanensis

Week	Percent of Original Lesion Diameter (mm ±SD)	
During Treatment	Ulcerated	Closed
1	105 ± 6	106 ± 8
2	113 ± 8	109 ± 17
3	118 ± 10	116 ± 14
4	122 ± 10	121 ± 26
Post-Treatment		
5	123 ± 9	119 ± 12
6	116 ± 10	129 ± 22

Figure 1

EFFECT OF TIOCONAZOLE ON *Leishmania braziliensis guyanensis* IN FEMALE BALB/c ByJ MICE



Mice were infected intradermally with $1x10^5$ amastigotes of *L. braziliensis guyanensis* and were treated for 3 weeks with Pentostam (160 mkd ◆···◆), Tioconazole (Tz) cream (1% ◇), Oral Tz (120 mkd ▽), Combination Tz (1% + 120mkd ▭), or with combination placebo cream and oral water (○).

TABLE 2

EFFECT OF TIOCONAZOLE UPON *Leishmania mexicana amazonensis*[α] INFECTIONS IN BALB/cByJ MICE

Treatment	Dose (mkd x 5)	Percent Original Lesion Diameter (mm ±SD)			Suppression
		Week -1	0	1	
Tioconazole					
Oral Only	120	115 ± 10	129 ± 13	129 ± 12	16
[β]Cream Only	1%	134 ± 31	128 ± 26	120 ± 27	22
Combination	120 + 1%	112 ± 11	118 ± 29	119 ± 8	23
[β]Placebo Combination	H_2O + 1%	133 ± 20	138 ±34	153 ± 19	--
[λ]Pentostam (Sb^V)	400				22

[α]Inoculum = 2×10^7 amastigotes

[β]Placebo cream = 20gm aquaphore in 30ml H_2O; Oral placebo = H_2O

[λ]Pentostam = 37.5% pentavalent antimony (Sb^V); Standard from Log Probit Plot, see also Table 3.

TABLE 3 Effect of Pentostam on *Leishmania mexicana mexicana* Amastigote- and Promastigote-Infections in Male BALB/cByJ Mice.

Parasite Stage	Pentostam Sb^0 mkd	Animals TOT	Animals EXP	Animals TOX	Lesion Wt. (mg) Cultures +/total	MEAN Lesion Area (mm^2) WEEKS 2-3	MEAN Lesion Area (mm^2) WEEKS 7-8	% Supp. 2-3	% Supp. 7-8	Toxic
Amastigotes	400 x 5	6	5	1	202 2/2	82	222	67	56	Yes
	800 x 5	6	4	2	79 2/2	60	169	76	66	Yes
	400 x 10	5	5	0	314 1/2	101	127	31	40	No
	200 x 10	4	4	0	642 2/2	126	177	14	17	No
	100 x 10	5	5	0	350 2/2	132	174	10	18	No
	400 x 15	5	5	0	166 nd	63	66	58	62	No
Promastigotes	400 x 15	4	4	0	147 2/2	88	108	62	74	No